# Early determinants of lung function in African infants.

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## Declaration

I, Diane Margaret Gray, hereby declare that this thesis is my own work, both in concept and execution, apart from the normal guidance received from my supervisors and contributions by those acknowledged.

Published manuscripts form part of this thesis. Any assistance received with study management, data collection, analysis and review from co-authors of the manuscripts is described below:

1. **Lung function in African infants: a pilot study** Gray DM, Willemse L, Alberts A, Simpson S, Sly PD, Hall GL, Zar HJ. Lung function in African infants: A pilot study. Published: Pediatric Pulmonology 2015; 50: 49-54.

**Contribution of student and co-authors:**

I developed the study methodology together with my supervisors, HZ and GH. I collected the lung function measures, completed the offline analysis and collated the data for analysis. The full draft of the paper including analysis was completed by myself. LW and AA assisted with collection of lung function measures, SS assisted with training and supervision of technical details of setup. PS contributed to study design. The co-authors, GH and HZ reviewed the manuscript and added conceptual and intellectual comment. All authors read the manuscript prior to submission.

2. **Respiratory Impedance in Healthy Unsedated South-African Infants: effects of maternal smoking** Gray D, Czovek D, Smith E, Willemse L, Alberts A, Gingl Z, Hall GL, Zar HJ, Sly PD, Hantos Z. Published: Respirology 2015; 20: 467-473.

**Contribution of student and co-authors:**

**I** developed the study methodology together with my supervisors, HZ and GH. The technique was developed by ZH, ZG and PS. ZH trained me in the measure and supervised data collection. I, LW and AA collected lung function measures. Data was analyzed offline by myself and DC. I completed the data analysis with assistance from ES. I completed the full initial draft of the paper. The co-authors, DC, ZH, PS, GH and HZ reviewed

the manuscript and added conceptual and intellectual comment. All authors read the manuscript prior to submission.

3. **Lung function and exhaled nitric oxide in healthy unsedated African infants Diane Gray, Lauren Willemse, Ane Visagie, Emilee Smith,** Dorottya Czövek, Peter D. Sly, Zoltán Hantos, Graham L Hall, Heather J Zar. Published: Respirology, 2015; 20: 1108-1114.

**Contribution of student and co-authors:**

I developed the study methodology together with my supervisors, HZ and GH. I, LW and AA collected lung function measures and completed offline data analysis. ZH and DC offered technical supervision of the FOT technique; GH offered technical supervision of the other techniques. Data analysis plan was devised and executed by ES and me. I completed the full initial draft of the paper. The co-authors, GH and HZ reviewed the manuscript and added conceptual and intellectual comment. All authors read the manuscript prior to submission.

4. **Title: Determinants of early life lung function in African infants** Diane Gray, Lauren Willemse, Ane Alberts, Dorottya Czövek, Polite Nduru MPhil, Aneesa Vanker, Dan J. Stein, Nastassja Koen, Peter D. Sly, Zoltán Hantos, Graham L. Hall, Heather J. Zar. Submitted for editorial consideration: Journal- Thorax; Date – June 2015

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I developed the study methodology together with my supervisors, HZ and GH. I, LW and AA collected lung function measures and completed offline data analysis. ZH and DC offered technical supervision of the FOT technique; GH offered technical supervision of the other techniques. DS devised the psychosocial methodology and NK supervised the collection of the psychosocial data. AV developed the environmental exposure methodology and analysis. Data analysis plan was devised and executed by myself and PN. I completed the full initial draft of the paper. The co-authors, GH and HZ reviewed the manuscript and added conceptual and intellectual comment. All authors read the manuscript prior to submission and commented/contributed within their area of expertise.

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**Contribution of student and co-authors:**

I developed the study methodology together with my supervisors, HZ and GH. I, LW and AA collected lung function measures and completed offline data analysis. GH offered technical supervision of the lung function techniques. DS devised the psychosocial methodology. AV developed the environmental exposure methodology and analysis. PS contributed to the original study design. Data analysis plan was devised and executed by LT and myself. I completed the full initial draft of the paper. The co-authors, GH and HZ reviewed the manuscript and added conceptual and intellectual comment. All authors read the manuscript prior to submission and commented/contributed within their area of expertise.

No part of the thesis has been submitted in the past, or is being, or is to be submitted for a degree at this or any other University.

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This thesis is presented for examination for the degree of PhD.

Signed:

Signed by candidate

Dated: September 2015

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## Abstract

Childhood respiratory disease remains a major contributor of morbidity and mortality globally and both paediatric and adult chronic respiratory illness is increasing in prevalence worldwide. This burden of respiratory disease is heaviest in low-middle income countries (LMIC), areas that have a high prevalence of known risk factors for respiratory disease, such us overcrowding, poverty and environmental air pollution. Much chronic respiratory illness has its origin in early life; further low lung function in infancy is associated with later respiratory illness. However, there is limited data on lung function in African infants despite a high prevalence of respiratory disease. Understanding the determinants of infant lung function will improve our understanding of prevention and management of respiratory disease.

This thesis aimed to describe lung function in South African infants from six weeks to one year and to investigate the impact of prenatal and early environmental exposures on lung function in infancy.

Infants enrolled in the Drakenstein Child Health Study, a multidisciplinary birth cohort study investigating the aetiology and outcome of early life lower respiratory tract infections (LRTI), were included. Seven hundred and forty one infants were enrolled from June 2012 to February 2015. Infants had lung function measured at six (4-10) weeks of age and one year (11-14 months). Measurements, made with the infant breathing via a facemask during natural sleep, included tidal breathing, exhaled nitric oxide, sulphur hexafluoride multiple breath washout and the forced oscillation technique. Information on antenatal exposures was collected using questionnaires and urine cotinine. Household benzene was measured antenatally.

The chapters of the thesis are presented as published manuscripts that describe the establishment of infant lung function for the first time in South Africa and the development of normative lung function in the first year of life. The final chapters investigate the impact of early life exposures, most notably LRTI, on lung function at six weeks and one year.

The thesis concludes that infant lung function testing is feasible in a community setting in

a LMIC like South Africa. Size, gender and ethnicity are important determinants of lung function. Lung function of South African infants is not well predicted by European reference equations, highlighting the importance of using population specific reference data when interpreting lung function tests.

The study identifies several factors including maternal smoking, maternal alcohol and household benzene exposure during pregnancy, associated with altered early lung function. In addition tracking of lung function in the first year of life is described in this cohort of African infants living in a high respiratory disease burden setting. The study identifies risk factors for impaired lung function at one year independent of low baseline lung function: LRTI, household smoke exposure and infant nutrition, factors amenable to public health intervention.

Given the fact that infant lung function tracks into later life and plays a role in chronic respiratory disease, preventing respiratory illness in young children, reducing exposure to environmental tobacco and maximising nutrition are key priorities in the strengthening of child respiratory health. Long-term study of lung function and respiratory disease in these infants is a priority in order to develop new strategies to strengthen child health.

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## Abbreviations

| | |
|---|---|
| BHR | Bronchial hyper-responsiveness |
| C | Compliance |
| $C_{rs}$ | Respiratory system compliance |
| CoV | Coefficient of variation |
| DOHAD | Developmental origin of health and disease |
| ETS | Environmental tobacco smoke |
| eNO | Exhaled nitric oxide |
| FOT | Forced oscillation technique |
| FRC | Functional residual capacity |
| $FRC_{MBW}$ | FRC measured by multiple breath washout |
| $FRC_{PLETH}$ | FRC measured by whole body plethysmography |
| *f*res | Resonance frequency |
| ILF | Infant lung function |
| I | Inertance |
| IQR | Interquartile range |
| LCI | Lung clearance index |
| LMIC | Low middle income countries |
| LRTI | Lower respiratory tract infection |
| MBW | Multiple breath washout |
| MSIP | Maternal smoking during pregnancy |
| R | Resistance |
| $R_{aw}$ | Airway resistance |
| $R_{int}$ | Interrupter resistance |
| $R_{rs}$ | Respiratory system resistance |
| RTC | Rapid thoracic compression |
| RVRTC | Raised volume raapid thoracic compression |
| SD | Standard deviation |
| SES | Socio-economic status |
| SF6 | Sulphur hexafluoride |
| $sC_{rs}$ | Specific respiratory system compliance (C related to FRC) |

| | |
|---|---|
| SOT | Single breath occlusion technique |
| $sR_{rs}$ | Specific respiratory system resistance (R related to FRC) |
| TBFVL | Tidal breathing flow volume loop |
| $t_{PTEF}/t_E$ | Ratio of time to peak tidal expiratory flow over total expiratory time |
| $V_{max}FRC$ | Maximal expiratory flow at FRC |
| $V_T$ | Tidal volume |
| WAZ | Weight for age z-score |
| WHZ | Weight for height z-score |
| $Z_{rs}$ | Respiratory system impedance |

Chapter 1

# Background and Aims

## Background

### 1. Introduction

Childhood respiratory disease remains a major contributor to morbidity and mortality globally, with acute lower respiratory illness a leading cause of death in children under five years.[1] Globally respiratory illness is the commonest reason for children to present to healthcare services and for admission to hospital.[2-5] The burden of respiratory illness is particularly high in low-middle income (LMIC) countries such as South Africa, with more than 90% of respiratory infection related deaths occurring in these areas.[6 7] African infants are particularly vulnerable with 50% of respiratory tract infection related deaths occurring in African countries.[8] Young children are particularly vulnerable with more frequent and severe disease in the first year of life. Severe lower respiratory tract infections (LRTI) are also common and severe in infants with malnutrition and HIV, both important paediatric health challenges in Africa.[8 9]

Early life respiratory infections are associated with later reduced lung function and chronic respiratory illness[10 11] and may play a role in the growing prevalence of chronic respiratory disease such as asthma and obstructive pulmonary disease.[12 13] Chronic respiratory illness is the fourth leading cause of death globally and is responsible for a large burden of global morbidity.[12 14] This burden is heaviest in LMIC, areas that have a high prevalence of known risk factors for respiratory disease, such us overcrowding, poverty and environmental air pollution.[15-18] Much of the chronic respiratory burden in later life, although exacerbated by personal factors such as smoking tobacco, has its origins very early in life, even antenatally.[19] Hence early life events play an important role in the development of respiratory illness in childhood and later life.

Objective measures of lung function can be used to delineate susceptibility to lower respiratory tract infections and the impact of these early life events on subsequent lung health. Given the high burden of respiratory disease in early life and it's association with later chronic respiratory disease measuring lung function could help inform appropriate new preventive and management strategies.

Several birth cohort studies from high-income countries have shown that early life lung function tracks through to adulthood. Therefore lung function achieved in infancy and childhood predicts lung function attained in early adult life and the point from which physiological lung function decline begins[20-22]; further low lung function in early childhood is a known risk factor for acute and chronic respiratory disease in later childhood as shown in several studies from high-income countries.[23-28]

Both antenatal and early life exposures affect the lung function achieved in childhood. Normal lung development is incomplete at birth, and continues during the early childhood years. During this time the lungs are vulnerable to damage if exposed to a noxious environment or exposure. Various factors have been identified as important determinants of early life lung function including infant growth[29 30], prematurity[31], early life respiratory tract infections[32], maternal disease such as asthma[33] or HIV infection,[34 35] environmental tobacco smoke[36] or other household air pollution[37 38]. This data is predominantly from high income settings; there is little data on infant lung function from LMIC and none from Africa. Lung function is a complex and coordinated system of central respiratory control and lung structure, which includes the airways, lung parenchyma, chest wall, respiratory muscles, peripheral and central receptors. It is likely that different exposures will affect the maturation and function of this complex system in different ways depending on the type, timing and combination of exposures in an individual.

In view of the large burden of respiratory disease in Africa, understanding the relative impact of antenatal and early life exposures on lung function in African infants is important if we are to develop new effective preventive strategies to strengthen respiratory health in childhood.

**2. Normal lung development**

The lungs undergo much growth and maturation during fetal and early postnatal life and hence are particularly vulnerable to deprivation or insult during this time.

The structural development of the lung starts in early fetal life with the airway branching mostly complete by 16 weeks gestation, comprising the conducting zone. After this

airways grow in size but not number. The respiratory zone begins to develop with the respiratory bronchioles from 16 to 24 weeks when the alveolar development begins. The alveolar do not appear until 28 weeks, with mature alveoli present only after 36 weeks gestation.[39] At birth only a small proportion (probably 15%) of adult number alveoli are present with substantial alveoli growth in number during the first 2-3 years of life,[40 41] These growth patterns have significant implications for our understanding of respiratory disease and subsequent lung health.

Alveoli continue to develop in volume and complexity during early life. The lung volume doubles in the first six months of life and triples by a year of age.[42] There is a dysynaptic growth pattern with the lung parenchyma growing relatively more than the airways during this time. Hence infants have a relatively large airway caliber to lung volume ratio compared to adults.[43] Lung physiology changes during the first year of life, with decreasing compliance and deformability of the chest wall and increase in size and development of the airways. Respiratory system resistance reduces with increasing airway size and lung volume. Similarly respiratory system compliance increases with increasing lung volume and chest wall stiffness.[44] The control of breathing, which includes the central medullary respiratory center, peripheral receptors, lung receptors and complex feedback mechanisms, develops during fetal life and matures in early post-natal life. Immature breathing control is associated with an irregular breathing pattern with considerable breath-to-breath variability and periodic breathing, especially noticeable in preterm infants.[45] Response to hypercapnia and hypoxia may also be variable or difficult to sustain in very young infants.[46] Factors affecting the brainstem, peripheral receptors and feedback mechanisms may alter the control of breathing in infants and children.

### 3. Measuring infant lung growth and function

To assess the impact of early life exposures or biological factors on normal lung growth and subsequent lung health, reliable measures of infant lung function are needed. For large epidemiological studies in healthy children these measures ideally need to be easy to perform, safe, have good repeatability and be able to accurately detect significant changes in lung function outcomes. Measurement of infant lung function has been used to assess normal lung growth and development[44 47], as an epidemiological tool to identify

risk factors for lung disease[23 36] and as an objective measure of infant and childhood lung disease diagnosis and progression.[48-50] However studies have used different methods and equipment making comparison between studies difficult. In addition many methods of testing are technically difficult and require sedation so have been limited to specialized testing centres and to small cohorts. However, more recently techniques such as the forced oscillation technique[51], multiple breath gas washout technique[52], exhaled nitric oxide[53] and tidal breathing volume measures[54] have been used successfully in unsedated infants during quiet sleep. A few recent birth cohort studies in high income countries have used unsedated testing in large prospective infant cohorts with adequate success.[24 55 56] However, there are currently no data on infant lung function in African children.

Different antenatal and early life exposures will affect either lung structural development as evidenced by reduced lung volumes, ventilation homogeneity, flow ratios and lung compliance; or control of breathing with effects on respiratory rate and flow ratios or effects on both. As a measure of airway inflammation, exhaled nitric oxide could add further information regarding mechanisms for lung function changes. Hence a comprehensive range of lung function measures are necessary to most accurately assess the effect of early life exposures on lung function development. Used longitudinally measurements can provide information on the timing and long-term impact of exposures on the respiratory system. The optimal tests to use depend on feasibility, safety and the ability of the test to assess the functional outcome studied. The currently available infant lung function tests are discussed below and summarised in **TABLE 1**.

### 3.1 Measurement of lung volumes

Lung volumes can be measured in infants using the whole body plethysmograph, inert gas multiple breath washout (MBW) or tidal volumes by simple measures of tidal breathing analysis. Plethysmography and MBW testing measure the functional residual capacity (FRC), a static lung volume that is a representative measure of lung growth and also important in the interpretation of volume specific lung function outcomes such as airway resistance and forced expiratory flows. The FRC is a balance between the chest wall and lung recoil , with changes in either affecting the FRC. Factors that decrease al-

veolar growth, cause lung atelectasis, decrease lung compliance or increase chest wall compliance will all lower the FRC. Airway obstruction with air trapping will raise the FRC. In infancy, due to the very compliant chest wall, infants control the FRC dynamically to prevent airway collapse. Hence in infancy the FRC variability is also influenced by control of breathing.

### *3.1.1 Plethysmography*

Infant plethysmography requires specialised equipment and highly skilled staff to accurately measure the FRC. Commercial equipment is available and recommendations for standardised equipment and technique have been published.[57 58] Adequate reference data are lacking however with most published data generated from small cohorts, using variable equipment and methodologies. There is no data from African infants.

Infant plethysmography has been used in prospective cohort studies assessing premorbid lung function and risk of respiratory disease in early life.[59 60] In a London infant cohort study Dezateux et al tested 118 sedated infants soon after birth, measuring the FRC using whole body plethysmography ($FRC_{pleth}$) with 91.5% success rate and measured 100 of them again successfully at one year of age.[60] They were able to use plethysmographic measures to demonstrate tracking of $FRC_{pleth}$ over the first year of life and assess the association of baseline $FRC_{pleth}$ on increased risk of wheeze episodes in the first year.[60]

This test affords the opportunity of monitoring changes in lung growth during a critical period of lung development. One of the advantages of this measurement technique over other measures of FRC such as inert gas MBW ($FRC_{MBW}$) is that it more reliably reflects total thoracic volume. $FRC_{MBW}$ measures the thoracic volume in communication with the airway opening and hence may under-report the lung volumes in the presence of gas trapping, that is $FRC_{pleth}$ may be higher than $FRC_{MBW}$ in the presence of obstructive lung disease.[61] Another advantage of plethysmography is that in addition to measuring lung volumes airway resistance and specific airway resistance can be measured simultaneously, see below. However, this technique requires the infant to be sedated and is resource intense, hence still remains a test for specialised laboratories.

### *3.1.2. Inert gas multiple breath washout*

An alternative method for measuring the FRC in infants is the inert gas MBW technique. This has gained favour in infant lung function testing given that it is non-invasive, can be collected during tidal breathing without special respiratory manoeuvres[62] and can be collected in unsedated infants.[56 63] This makes it more feasible for testing large cohorts of healthy infants. Commercial equipment is available and international consensus recommendations for collection and interpretation of this technique have been recently published.[62]

A prospective Swiss birth cohort measured sulphur hexafluoride (SF6) MBW in 352 healthy unsedated infants (age mean±sd 5.1±0.8 weeks) with a success rate 57% and an intra-individual coefficient of variation for FRC of 6.3% (IQR 4.4 to 8.2).[56] In 64 unsedated healthy term infants at birth tested with the same methods and equipment, Hulskamp et al had a success rate of 90%.[63] SF6 MBW testing has been used successfully in unsedated infants to assess the impact of infant demographics and early life exposures on FRC.[56 63 64] An alternative, the nitrogen MBW techinique requires the breathing of 100% oxygen which has been shown to alter the infant breathing pattern and is not recommended for use in infants.[65 66] The MBW testing technique has been able to detect the impact of prematurity[63] and cystic fibrosis[64] on early life $FRC_{MBW}$, adding to current understanding of the effect of early life events or disease have on normal lung growth.

In summary the inert gas MBW testing can be successfully undertaken in unsedated infants during quiet tidal breathing. These measures are sensitive enough to detect the impact of early life exposures. An additional advantage of this test is the ability to simultaneously measure indices of ventilation homogeneity such as the lung clearance index (LCI), see below.

### *3.1.3 Tidal breathing volumes*

Tidal volume can be relatively easily measured during tidal breathing. The advantage of tidal breathing flow volume loop (TBFVL) measurement is that it requires no respiratory manoeuver, can be achieved in unsedated infants during quiet tidal breathing and has been used in many infant cohort studies facilitating comparison between groups.[23 29 55 56 67 68]

However lung volumes and flow measured during tidal breathing, though relatively simple to collect, are less simple to interpret. Tidal volume is a function of multiple parts of the complex respiratory control mechanism,[69 70] and tidal breathing parameters probably reflect both respiratory mechanics and control of breathing. For this reason changes in tidal volume are a less specific measure of lung growth than the FRC. Standardised recommendations on tidal breathing measures have been published and commercial equipment is available.[71] Although reference data for infant tidal breathing have been published,[56 70 72-74] these were collected from mostly small samples and in variable wake states (natural sleep,[56 73] sedated[70] or awake[74]) from Caucasian infants, and hence anyone using these measures should ideally produce reference data from their own population. There is no data from African infants.

Many cohort studies have used TBFVL to measure the impact of early life demographics and exposures on tidal volume and flows and association of these measures with subsequent wheeze.[23 73-75] These studies have shown value in documenting association of low lung function with subsequent wheezy respiratory disease[23 73 75] and the reduced lung function in infants whose mother smoke during pregnancy.[74 76 77] Tidal breathing measures have also been used in longitudinal studies to asses lung function over time.[67 78] There is much discussion about what are the most valuable measures and how useful these measures are in the detection of lung function impairment.[70] The most commonly reported measures are respiratory rate, tidal volume, time to peak tidal expiratory flow ($t_{PTEF}$), total expiratory time ($t_E$) and the ratio $t_{PTEF}/t_E$. The latter outcomes are discussed further below. Tidal volume increases in a non-linear manner with somatic growth in the first year of life and then continues to increase with somatic growth through childhood.[79]

The advantages of this test include the ease of collection and the ability to use these measures longitudinally. In addition they represent the result of the complex control of breathing and, although not specific, the various outcomes can provide information about respiratory control e.g. respiratory rate, minute ventilation and inspiratory flow ratios; lung growth e.g. tidal volume and airflow obstruction e.g. expiratory flow ratios. However, when interpreting these outcomes, the non-specific nature of these measures needs to be taken into account.

### 3.2 Measurement of air flow

Air flow can be measured in infants either as forced expiratory manoeuvers, infant spirometry, or as tidal breathing flow volume loops. Infant spirometry measures maximal flow at forced vital capacity, forced expiratory volumes in unit time and expiratory flow at percentage forced vital capacity (FVC). In this way they are similar to measures of forced expiration collected with standard paediatric and adult spirometry, a commonly used lung function tool in older children and adults. Small lung volumes and small or obstructed airways would result in reduced expiratory flows. Inspiratory and expiratory flows measured during tidal breathing have also been used to assess lung function and airway obstruction in infants. Tidal breathing measures are however less sensitive measures of air flow limitation compared to forced manoeuvres as the tidal measures are affected more by control of breathing than the forced manoeuvres.

#### *3.2.1 Infant spirometry*

Measurement of forced expiratory flows from total lung capacity to residual volume is the most commonly used clinical assessment of lung function in co-operative children and adults. These manoeuvres are possible in spontaneously breathing sedated infants using specialised techniques. This can be done using the rapid thoracic compression (RTC) manoeuvres either from tidal volume[80] or over a larger volume by raising lung volume above tidal inspiration before the thoracic compression, raised volume RTC (RVRTC). [81] The RTC squeezes out air at the end of tidal or raised volume inspiration by a compression of an inflatable jacket which applies an external pressure to the chest wall and abdomen. The technique requires sedation of infants as well as a highly skilled technician with special knowledge of respiratory physiology in order to obtain accurate and safe infant measurements. Standardised methods have been published, but adequate reference data is lacking.[80 81]

These forced manoeuvres rely on the physiological concept of flow limitation, the point at which expiratory flow becomes effort independent. The partial flow volume curves obtained from tidal RTC are most commonly quantified by the maximal expiratory flow at FRC ($V_{max}$FRC). This measure represents the function of smaller, peripheral airways.[82] However the disadvantage of this test is the lack of a reliable baseline volume for the

measurement of $V_{max}$FRC. This limitation led to the development of the RVRTC technique which uses a predetermined lung volume via a given inflation pressure, allowing the calculation of volume-time parameters. The most commonly reported measures are FVC, and forced expiratory flows (FEF) at 25%, 75% and 85% of FVC and the average flow between 25-75 % of FVC ($FEF_{25-75}$). The forced expiratory flow measured in this way has comparable within-subject variability to that obtained by standard spirometry in preschool children.[83]

Many birth cohort studies have used the RTC [23 26 30 32 75 84-86] and more recently the RVRTC[87 88] technique to measure sedated infant lung function and to assess the impact of early life factors on lung development and respiratory illness in the first few years of life.

The main advantage of this test is the ability to measure forced expiratory flows and volumes at FVC, measurements that are commonly used to assess pulmonary function in older children and adults. In addition this technique is a more sensitive measures of airway obstruction than tidal breathing expiratory flows.[68] However the disadvantage of this technique is the requirement for complex equipment and need for sedation. Differences across laboratories in technique of raising lung volume have led to limitations in comparing data across sites. The need for sedation reduces its applicability to epidemiological studies of healthy infants. Although more sensitive than tidal breathing measures, neither technique is able to separate the contribution of airway size and mechanics to lung function outcomes and like other measures of lung function are unable to definitively identify the site of lung function changes.

### *3.2.2 Tidal breathing measures of flow*

As discussed above, measures of tidal inspiratory and expiratory flows have been commonly used as measures of infant lung function in epidemiological and clinical studies.

Reduced expiratory flow ratios, most commonly the peak tidal expiratory flow over the total expiratory time ($t_{PTEF}/t_E$), have been associated with infant respiratory disease and early childhood wheeze.[23 73 85] The $t_{PTEF}/t_E$ is decreased in children with wheezing disorders,[72] acute bronchiolitis[89] and after bronchoprovocation testing.[90] Factors affecting the $t_{PTEF}/t_E$ include the elastic recoil pressures of the respiratory system, the resistance

to flow in the airways and control of breathing, particularly in early life where expiratory breaking and stretch receptor reflexes interact to control the end-expiratory lung volume and optimise ventilation.[91] Hence this measure, though related to airway obstruction is also affected by other aspects of breathing control.

However its frequent use in prior epidemiological studies and the fact that it can be completed safely in unsedated infants, makes measurement of tidal breathing outcomes a useful tool for longitudinal assessment of a healthy birth cohort in a community setting.

### 3.3 Measurement of lung mechanics

Respiratory mechanics describe the resistive, elastic and inertive forces that need to be overcome for ventilation to happen efficiently. The mechanics are described by the relationship between the changes in pressure, flow and volume during breathing and include the mechanical properties of the airways, lung tissue and chest wall. Mechanical properties are usually described by compliance (C), resistance (R) and its reciprocal conductance (G). If these measures are related to the lung volume at which they were measured, it is termed specific resistance (sR) or compliance (sC). Respiratory mechanics may be measured in various ways. These include passive measurements i.e. when the respiratory muscles are at rest such as during apnoea or active measurements when the muscles are actively contracting. An example of passive measurements is the occlusion technique and active measurements, the interrupter, plethysmographic and forced oscillation techniques. Different tests describe different aspects of respiratory mechanics. For example, by measuring changes in alveolar pressure airway resistance ($R_{aw}$) can be measured such as is done by plethysmography. Respiratory system resistance ($R_{rs}$) can be calculated as change in pressure at the airway opening e.g. single occlusion technique. However, no commonly used techniques are able to distinguish between the relative contributions from lung tissue, intra- and extra thoracic airways.

#### *3.3.1 Single breath occlusion technique (SOT)*

The SOT measures the alveolar pressure during a brief occlusion of the airway and apnoea induced by the Hering-Breuer reflex, so that the respiratory muscles are relaxed. The pressure measured reflects the elastic recoil pressure of the respiratory system at

any given volume. This technique measures respiratory system compliance ($C_{rs}$), resistance ($R_{rs}$) and time constant ($C_{rs}$ x $R_{rs}$). Standard recommendations for applying SOT have been published[92] and commercial devices are available, although adequate reference data is lacking. SOT has been used in unsedated infant cohorts.[24 55] The Wheezing Illness Study Leidsche Rijn (WHISTLER) study, a birth cohort study investigating the determinants of early life wheezing, collected quality measures of respiratory C and R in a large group of infants tested at average 4.6±1.3 weeks.[93] They had a 73% success rate in unsedated SOT testing and were able to show associations of baseline lung function with subsequent disease risk.[24]

The SOT has the advantage of being relatively non-invasive and possible in unsedated infants. It contributes information regarding the elastic and resistive properties of the respiratory system. It has been used successfully as an epidemiological tool in healthy unsedated infant cohorts. Disadvantages are that the technique relies on the assumption of the lung as a single compartment model. This is particularly problematic in disease states. It also assumes near instantaneous equilibration of pressure at the mouth during the brief occlusion. This may not be the case in severe airway obstruction or in very young infants who have exaggerated respiratory reflexes, and hence theoretically may not respond with the Hering-Breuer reflex. Similarly prolonged expiratory breaking may delay this equilibration and lead to inaccurate measures.[94] In addition, although it has been used as an epidemiological tool, it may not be as sensitive a discriminator of clinical disease as plethysmographic $R_{aw}$.[95]

### *3.3.2 Interrupter technique*

Interrupter resistance ($R_{int}$) is collected by measuring the flow and pressure at the airway opening. It uses a shutter-device to briefly interrupt airflow. The change in pressure at the airway opening during the occlusion is used to estimate the driving pressure across the airway at the time of the interruption.[96] The $R_{int}$ is hence a representation of the airway resistance ($R_{aw}$), but does contain elements of chest wall and lung tissue resistance, hence $R_{int}$ is usually higher than plethysmographically measured $R_{aw}$.[97] The technique also relies on the assumptions of instantaneous shutter closure and equilibration of pressure at the airway opening with alveolar pressure. Recommended equipment and testing

standards have been published, but internationally applicable reference data is lacking.[98] Although used in preschool children this technique has been less used in infants. It has however been successfully used in unsedated infants in a prospective birth cohort study measuring lung function at six weeks of age in healthy infants.[56] This study had 72% success rate in obtaining successful measures in unsedated infants, but with a high variability, median intrasubject coefficient of variation of 19% (IQR 14.7 – 25.3).

The primary advantage of the interrupter technique is that it is non-invasive, can be completed during unsedated sleep during tidal breathing; the equipment is portable and commercial machines are available. However the dependence of this test on instantaneous equilibration of pressures during the shutter closure makes it potentially inaccurate in the presence of airway disease and could lead to a systematic bias in results between disease and healthy participants. In addition, like with many infant techniques for measuring respiratory system resistance, the inclusion of the nasal passages and cheeks can further reduce accuracy. Paying special attention to cheek support and equipment standards is important.[99]

### *3.3.3. Forced oscillation technique (FOT)*

The FOT as a measurement of respiratory mechanics has recently gained in popularity particularly because it can be done quickly during tidal breathing and requires no respiratory manoeuvre and minimal patient co-operation. The underlying principle of the FOT is the application of an external driving signal to the respiratory system and the measurement of the respiratory system response to that applied force. From this response the respiratory impedance (Zrs) is derived, this is the frequency-dependent relationship between pressure and flow. Impedance describes the resistive (pressure changes in phase with flow) component and the reactive component (pressure changes out of phase with changes in flow) of the respiratory system. The reactive component describes the elastic properties of the tissues and the inertial properties of air flow in the airways. The signal or sound wave generated from a loudspeaker is applied as input impedance at the airway opening. Although much infant work using a low frequency FOT (LFOT) suggested that the frequency dependence allowed one to partition the respiratory system response from tissues and airways this has not been consistently shown.

Most commercially available equipment uses a medium frequency signal that measures the resistance and reactance of the respiratory system as a whole and cannot be used to partition the response between tissues and airways. There is currently no commercially available equipment suitable for use in infants. Recommendations for data collection, quality control and reporting have been published.[100]

FOT has already been well validated for use in children and adults and is able to discriminate between health and disease in a number of paediatric diseases including asthma [101 102], bronchopulmonary dysplasia[103] and cystic fibrosis. [51] However FOT in infants has not been routinely used. More recently a FOT technique modified for use in unsedated sleeping infants has been developed.[104] This modification uses a medium range of frequencies (8 to 48 Hz) and provides estimates of respiratory system resistance, compliance and resonant frequency; as such this test reflects the growth and maturation of both the airways and lung parenchyma. This technique has not been used in infant epidemiological studies, but if sensitive enough to detect the impact of early life exposures on lung function and growth would be an ideal tool to longitudinally measure lung mechanics during early life through to adulthood.

FOT has the advantage of being a non-invasive tool and is particularly useful in the measurement of lung function in infants and children who are unable to co-operate with standard lung function measures, which require controlled respiratory manoeuvers. It is collected during tidal breathing, removing the need for breathing interruption or sedation. Moreover the frequency dependence and the possibility of breath-by-breath analysis of data, provide the potential to more sensitively gather information regarding respiratory system structure and effect of respiratory disease.

The main disadvantage of this technique in infants is the inclusion of the upper airway in the measurement. The measured impedance therefore contains contributions from the upper airways and nasal passages which have high resistance[105] and the upper airways act as a shunt which absorbs a certain amount of the applied signal. This needs to be taken into account when collecting and interpreting results. [106]

### *3.3.4 Plethysmography airway resistance*

The resistance of the airways ($R_{aw}$) can be measured by whole body plethysmography which measures the changes in box pressure as representative of alveolar pressure and relates this to changes in flow at the mouth. The resistance measured and its reciprocal conductance represents that of the airway resistance and conductance. When related to the lung volume measured ($sR_{aw}$ and $sG_{aw}$) it is a measure of the effective airway size corrected for lung size and thereby somatic growth.[107] These measures are taken during quiet tidal breathing and hence non-invasive, although do require sedation. Recommendations for testing standards and commercial equipment are available, but appropriate reference data is lacking.[57] This technique has been used in birth cohort studies from high income countries in sedated infants to describe airway resistance and the impact of early life exposures on respiratory mechanics over time.[59 60]

The main advantage of the plethysmographic measurements are the rapid non-invasive measurement of airway resistance that can be related to simultaneously collected FRC. However, the equipment is expensive and bulky and hence not a portable field tool. It is also not suitable for very small or sick infants, such as preterm infants or infants with respiratory infections. Testing requires very experienced and skilled staff e.g. careful attention has to be paid to pressure changes due to changes in temperature and humidity during the respired air throughout the testing, adding to the technical difficulties of achieving accurate results. In addition testing requires sedation which reduces its feasibility in large cohort studies of healthy infants.

## 3.4 Measurement of ventilation homogeneity

Another important functional property of the lung is intrapulmonary gas distribution and mixing, which is relatively evenly distributed or homogenous in the healthy lung. This can be measured in infancy using the MBW technique.[108] Using indices measured from the washout curve, ventilation inhomogeneities can be calculated. The most commonly used index is the lung clearance index (LCI), but other more sophisticated indices include the moment ratios (M1/M0 and M2/MO),[109] the alveolar gas dilution number[52 109] and the mixing ratio (the ratio of actual and ideal breaths required to reduce the starting gas concentration to the predefined end point)[110]. The indices of ventilation homogeneity are

independent of age and hence potentially useful longitudinal markers of lung function.

### *3.4.1 Lung clearance index*

The MBW technique can be used to measure the efficiency and distribution of ventilation.[62] The most commonly reported measure of ventilation homogeneity is the lung clearance index (LCI) which has been successfully used in infants.[52 63 111 112] An increased LCI is evidence of impaired gas mixing or ventilation distribution and is a sensitive measure of peripheral airway obstruction and early lung damage in infants and young children.[111 113 114] The lung clearance index is defined as the cumulative expired volume needed to lower the end-tidal marker gas concentration to 1/40$^{th}$ the starting by the FRC, that is the number of FRC turnovers needed to clear the marker gas from the lungs. Consensus recommendations have been recently published[62] and commercial equipment for infant testing is available, however no internationally applicable reference data are currently available. Unsedated MBW testing in infants has been successfully undertaken in cohort studies.[56 63]

An advantage of the LCI is that this is measured during quiet tidal breathing and so can be completed in very young children. In addition this measure, as a function of the FRC, changes little through life and can potentially be used to track lung function from infancy through childhood to adulthood. Another advantage is the potential for this test to detect early peripheral lung disease and has been shown to be a more sensitive measure of lung disease than measures of expiratory flow in children with cystic fibrosis.[113] A disadvantage of the test is that it takes time and needs to be collected during quiet breathing free of sighs or respiratory pauses, hence is less feasible in unsedated infants than measures of lung mechanics for example. The MBW test is resource heavy; equipment is expensive and requires skilled operators to ensure accurate collection and analysis of data.

## 3.5 Measurement of airway inflammation

Assessing airway inflammation is important in investigating the underlying pathophysiology of many respiratory diseases. In addition measuring markers of airway imflammation may assist in monitoring respiratory disease and treatment in children with inflammatory lung disease such as asthma. Methods used to assess airway inflammation include

measurement of blood, urine, sputum, exhaled breath condensate and exhaled breath nitric oxide. Products of inflammatory cells such as eosinophils can be measured in the blood (e.g. eosinophilic cationic protein) and urine (e.g. eosinophil protein X) and have been associated with symptom control in asthmatic children.[115 116] Sputum analysis for inflammatory cells, in particular eosinophils, has also been associated with asthma symptom control in adults and children,[117 118] and has identified non-eosinophilic inflammation as important in some patient sub-groups.[119] In infants this requires sputum induction. Exhaled breath condensate (EBC) is a non-invasive method of collecting a respiratory specimen. EBC contains a large number of mediators associated with respiratory disease and hence a potentially useful tool to measure the inflammatory mileu of the respiratory tract.[120] One of the most studied and measured exhaled inflammatory markers is nitric oxide which can be collected during tidal breathing in infants.[53]

### *3.5.1 Exhaled nitric oxide*

Analysis of exhaled breath may play a role in understanding disease mechanisms, aid in diagnosis and in clinical management of chronic respiratory disease. Exhaled nitric oxide (eNO) is a measure of eosinophilic airway inflammation. In infants it has been shown to be raised in infants of atopic mothers [121], those with a history of recurrent wheeze[122] and those exposed to air pollution.[38] Exhaled NO levels may also be affected by age, gender, ambient NO, diet, smoking habits and disease states such as cystic fibrosis and primary ciliary dyskinesia.[123]

Tidal breathing measures of eNO have been successfully measured in unsedated infants.[53 122] The measurement is a quick and non-invasive test that can be collected during quiet tidal breathing in unsedated infants. It adds novel information to understanding lung health that is not provided by other measures of lung growth and function; hence it may add information regarding the mechanism behind loss of lung function, particularly in wheezy infants. A disadvantage with tidal breathing measures in infants using a facemask is that it includes eNO from the upper as well as lower airways. As nasal eNO is usually higher than that from the lower airways this can raise the measurement.[124]

## 4. Antenatal and early life determinants of infant lung function

In response to the growing understanding of the early origins of disease, there have been a number of studies investigating the antenatal and early life determinants of infant lung function. Most of this work has taken place in high-income settings, with minimal data from LMIC and none from African infant populations.

Adverse events during pregnancy may have direct effects on the growing fetus e.g. maternal smoking is associated with reduced infant birth-weight[125]; or may prime the infant's body to maladaptive responses to events in later life e.g. in utero maternal smoking and increased postnatal responses to allergen exposure.[126] The latter falls within the developmental origins of health and disease (DOHAD) hypothesis, first described by Barker in his epidemiological studies which showed that areas with a high infant mortality also had a high incidence of adult death from diseases such as respiratory disease.[127 128] The DOHAD hypothesis suggests that an in-utero event reprograms an individual to survive the in-utero environment with consequent increased risk for systemic disease in later life.[129]

Trans-generational epigenetic effects have also been described in respiratory disease. For example in utero smoke exposure is associated with a higher risk of asthma developing two generations later. A grandmother who smokes increases the chance that her grandchild will develop asthma, even if the child's mother does not smoke.[130] Other important exposures have a similar effect e.g. exposure to air pollutants or alcohol. This adds another complex layer to understanding the determinants of respiratory disease.

A number of birth cohort studies have investigated the effect of in utero exposures on early life lung function, identifying factors associated with lung function achieved soon after birth and at one year. The various infant cohort studies are summarized in **TABLE 2**, including details of sample size, participants and lung function testing. Because infant lung function was until recently restricted to highly specialized units and equipment and testing methods were not standardized, many early studies had small sample sizes and used variable techniques and equipment making comparisons between studies difficult. However, many factors have emerged as important in determining early lung function achieved.

### 4.1 Infant growth

Children and adults who were small at birth have lower lung function [131 132] and increased risk of respiratory disease and death in later life.[10 133] Infant lung function measures are dependent on body size, therefore it is expected that larger infants would have larger lung volumes with improved flows and lung mechanics.[29] Gestational age adjusted weight at birth, a reflection of fetal growth, is however independently associated with lung function in early life.[29 30 33 134] Dezateux et al, in a population based cohort of full-term infants with six week lung function measures, found infants who were small for gestational age had lower lung volumes, flows and respiratory system compliance at 6 weeks compared to infants born appropriate for gestational age, an effect independent of weight and length at test.[33] This effect persisted during the first year of life.[29] This suggests that factors causing impaired fetal growth also impair lung and airway development and possibly also postnatal programming in line with the DOHAD principle.

However being large for gestational age may also be a risk factor for impaired lung function. Infants with a high birth weight (>4.5kg) enrolled in a population based Canadian cohort, had an increased risk of hospital visits for asthma in the first 10 years of life[134] suggesting that being overweight at birth may affect lung mechanics in early life. In addition postnatal weight gain has been negatively associated with normal lung function in early life. Increased postnatal weight gain has been associated with lower forced expiratory flows and lung volumes[30 33 135] This increased postnatal weight gain has also been associated with increased wheezing and doctor diagnosed asthma in children up to three years of age.[135] In a meta-analysis of 31 birth cohort studies including over 147 000 infants followed to school age, increased postnatal weight gain increased risk of preschool wheeze and doctor diagnosed asthma at school age.[136] This suggests a sustained effect of weight gain on lung mechanics through childhood and is a potentially preventable risk factor for impaired lung function.

### 4.2 Prematurity

Prematurity is associated with decreased lung function at birth with persistence through early life and is associated with low lung function and chronic respiratory illness in older children and adults.[137-139] The prematurity-associated reduced lung function is thought to

be due to a combination of immature lung development (alveolarisation, vascular) and postnatal events and exposures such as oxygen therapy and ventilation induced lung damage. Even in infants without a history of neonatal respiratory disease however, lung growth is reduced, independent of birthweight.[139] Lung function abnormalities in very premature infants <32 weeks are well documented.[139-143] In addition late preterm infants (33-36 weeks gestation) have also been shown to have reduced early life lung function compared to full term infants. [144 145] Strategies to prevent preterm birth, judicious use of corticosteroid therapy and surfactant and optimising postnatal care are important strategies to reduce early life lung function impairment.

### 4.3 Maternal smoking during pregnancy

One of the most consistent and much studied prenatal exposures is maternal smoking during pregnancy (MSIP), which has convincingly shown adverse effects on early lung growth and function in both human and animal models.[36] Many studies have tested lung function at a few weeks of age and hence described the effect of both in utero and early life passive smoke exposure.[60 74 85 86 146 147] However, studies assessing in utero exposure alone with lung function at birth have also shown significant impairments of lung function.[76 77 148] This adverse effect occurs not just during exposure in the last months of pregnancy but also with early pregnancy exposure, as has been shown in studies in preterm infants. [77]

The effect of maternal smoking on lung function is independent of reduced somatic growth. Adjusted forced expiratory flows are reduced in infants' whose mothers smoked during pregnancy. This includes reduced maximal expired flow at functional residual capacity ($V_{maxFRC}$)[26 77 149] and forced expiratory flows when 75% of forced vital capacity is expired ($FEF_{75}$).[87 146 150] These are measures of peripheral airway obstruction. Effects of maternal smoking on respiratory system resistance and compliance have also been described. Lodrup-Carlsen et al testing a large cohort of healthy infants using the single breath occlusion technique, found a lower compliance in female infants whose mothers smoked during pregnancy compared to those whose mothers did not.[74] Similarly in an English cohort of healthy full-term infants tested at 2.5 days of life, infants exposed to MSIP had reduced compliance.[148] Others have found no effect of MSIP on $C_{rs}$ but a slight

increase in R.[60 93] Measurement of passive respiratory mechanics may be a less sensitive measure of smoking effect on lung function than the forced expiratory maneuvers, but taken together support the findings of animal studies describing altered airway development with lung hypoplasia in nicotine exposed fetuses.[151 152]

Tidal breathing measures have also been used to assess the impact of MSIP on infant lung development. The most reported measure is the ratio of the time to peak tidal expiratory flow over total expiratory time ($t_{PTEF}/t_E$). Stick et al assessed lung function in the first few days of life (mean 62 hours) prior to discharge from hospital to determine any effect of in utero smoke exposure. They found the $t_{PTEF}/t_E$ was reduced in smoke exposed infants and that the effect was stronger in mothers who smoke >10 cigarettes per day, suggesting a dose effect.[76] Reduced $t_{PTEF}/t_E$ has also been found in other cohorts investigating in utero smoke exposure[77] and MSIP together with early postnatal passive smoke exposure.[74] This ratio may reflect a smaller airway caliber. However the $t_{PTEF}/t_E$ is the result of a complex interaction between mechanical and neurological control and an altered ratio could reflect either or both mechanically reduced airway caliber and altered control of breathing.[153 154] In addition to effects on lung mechanics, infant respiratory control may also be affected by MSIP – with blunted response to hypoxia and increased risk of sudden infant death.[155-157] Hence the effect seen in reduced $t_{PTEF}/t_E$ may reflect both the structural effect of smoke exposure on airway development and the modified control as evidenced by altered response to hypoxia.

Taken together in utero smoke exposure appears to cause structural changes in the developing lung, which persist. Exposed babies have smaller airways at birth and hence are more likely to wheeze with inter-current infections. They may have altered responses to hypoxia making them vulnerable to poor outcome with respiratory tract infections and increased risk of sudden infant death. Post-natal exposure to household cigarette smoke is an independent risk factor for impaired lung function in most studies.[59 85 158] and may also exaggerate the effects of in utero exposure and predispose to more frequent, severe early life infections.

### 4.4 Maternal asthma and/or atopy

A family history of asthma and/or atopy has been investigated as a predictor of early life lung function. A maternal history of asthma is known to increase the risk of wheezing episodes in early life, either through inherited vulnerability to bronchial hyper-responsiveness,[85] reduced low lung function at birth[60] or altered responses to infectious allergen exposure.[126] Martinez et al described the early life patterns of wheeze in a longitudinal cohort of USA children followed from birth through to six years and correlated baseline lung function, serum IgE, maternal asthma and frequency of wheezing episodes through the first 22 years of life.[20 159] They found that a maternal history of asthma was not associated with low lung function in early life, but that children of asthmatic mothers were more likely to have a history of persistent wheezing through the first years of life and reduced lung function at six years.[159] However, others have noted an association of family history of asthma and low lung function at birth.[60 76] In 101 infants followed in a UK prospective birth cohort and who had lung function before 13 weeks of age, a family history of asthma was associated with decreased specific airway conductance (1.98 $s^{-1}.hPa^{-1}$ vs. 2.6 (1.0) $s^{-1}.hPa^{-1}$; 0<0.05).[60] In the Western Australia Pregnancy Cohort study, a family history of asthma was associated with a decreased $t_{PTEF}/t_E$ at one month in 461 infants.[76] Others have noted an association of familial asthma and bronchial hyper-responsiveness.[160] Young et al assessed bronchial hyper-responsiveness (BHR) with histamine challenge at one month of age and found infants with a family history of asthma had an increased BHR compared to infants who did not have this history (PC40 0.78 $g.L^{-1}$ vs. PC40 2.78 $g.L^{-1}$ respectively).[160] Infants born to atopic parents and who have low lung function in early life have an increased risk of wheezing illness and asthma.[26 88] Elevated levels of exhaled nitric oxide (eNO) are associated with atopy. Latzin et al measured eNO in infants in a Swiss birth cohort soon after birth and prospectively followed them for respiratory symptoms in early life.[121] Infants of atopic mothers with high eNO levels were more likely to wheeze than those who did not have raised eNO. This suggests that there is early and pre-symptomatic activation of atopy associated airway inflammation in infants with a genetic predisposition. In at risk children (both parents atopic) prospectively followed in the Copenhagen Prospective Study on Asthma in Childhood (COPSAC) study, those that were subsequently diagnosed with asthma at 7 years had reduced expiratory

forced expiratory flow at 50% forced vital capacity and BHR as neonates compared to at risk infants who did not go on to develop early childhood asthma.[88]

In summary, maternal history of asthma and atopy is associated with increased risk of wheeze in later life. Although it is associated in some studies with reduced early lung function this association becomes more consistent in later childhood with effect increasing over time.[88] Taken together this suggests that maternal atopy infers an inherited risk for bronchial hyper-responsiveness and wheeze, which becomes more apparent later in childhood. The impact of asthma and atopy on early lung function in African populations has not been studied.

### 4.5 Gender

Previous studies have shown male infants to have reduced early life lung function as compared to female infants.[23 85 158 161-163] These differences include higher respiratory system resistance, lower compliance and reduced expiratory flows in male compared to female infants. Hanrahan et al. investigated healthy infants at 2 to 6 weeks with the SOT and reported that male infants had a significantly higher R (0.083 vs. 0.079 cm-$H_2O$.s.$mL^{-1}$; p=0.003) but a non-significantly lower C (5.4 vs. 5.54 mL.cm$H_2O^{-1}$; p=0.37) compared to female infants.[158] Stocks et al. tested preterm infants at 5 to 39 days with the SOT and multiple occlusion technique; female infants had a non-statistically significant lower resistance than males (95%CI: -2.93; p=0.2), and significantly higher time to peak expiratory flow over total expiratory time.[161] This is consistent with other reports of low lung function in male infants[77 85 158 163 164], suggesting that male infants may have a less mature respiratory system at birth with smaller airways and functional residual capacity compared to female infants. This difference in lung function between male and female infants tracks through the first year of life.[85] Although not all studies have shown male sex to impact normal lung growth, responses to post-natal insults are modified by sex.[25] This lower early lung function in boys may be one factor contributing to the higher rate of respiratory disease amongst boys in childhood.[165]

### 4.6 Ethnicity

Ethnic differences in lung function in older children and adults are well established. Peo-

ple of Black African ethnicity having lower forced volumes and expiratory flows compared to Caucasians after correcting for anthropometry and sex, a difference thought to be related to trunk leg ratio as lung function based on sitting height is similar between races.[166 167] Ethnic differences in infant lung function have been previously reported.[77 107 164 168 169] Full-term Black African infants have a lower nasal resistance[169] and increased airway conductance[107] compared to Caucasian infants, which may be related to differences in anatomical structure. Prolongation of $t_{PTEF}/t_E$ [77 164] and higher forced expiratory flows[77] in Black Afro-Caribbean compared to Caucasian European preterm infants has also been previously described, suggesting a different early pattern of breathing in African infants. Ethnic differences in lung function at birth and later in life, may relate to a number of factors including anatomical structure and alterations in maturation of respiratory control. Although early life differences may be transient, defining the effect of ethnic group on a population and taking this into account when interpreting lung function measures is important. There is very little lung function data published for any Southern African infant or paediatric populations, hence collecting such information will add to our understanding of normal lung growth in these populations and how this may differ to what is already known in other populations.

### 4.6 Air pollution

There are very few data on the impact of antenatal exposure to air pollution on infant lung function. Maternal exposure during pregnancy to particulate matter less than 10 microns ($PM_{10}$), nitrogen dioxide and ozone has been associated with increased minute ventilation and exhaled nitric oxide in the infants.[38] Given the association of air pollution with respiratory disease, understanding the impact of antenatal exposure on early lung function is an important area requiring further research.[170] Postnatal exposure to air pollution has been associated with decreased lung function in children[171] and chronic exposure with decreased lung growth.[172 173] This includes exposure to nitrogen dioxide, particulate matter, ozone and volatile organic compounds including benzene.[171 172] Recent evidence suggests that reducing exposure to air pollutants can ameliorate the impact on lung growth,[174] highlighting the need for public health interventions targeting air pollution particularly in high risk communities.

### 4.7 Wheezing and respiratory tract infections

Children with wheeze, with or without respiratory tract infections, in the first few years of life have lower lung function at one year and two years, but this effect appears to be mediated by pre-existing low lung function.[27 59 67 175] Longitudinal studies of infant lung function have shown low lung function to precede wheezing episodes.[23 42 176 21 25] Lung function in infants that wheeze, tracks during the first few years and wheezing episodes do not appear to impair normal lung growth.[59 67]

The association between early life lower respiratory tract infections (LRTI) and subsequent decreased lung function and chronic respiratory symptoms has been less well studied. LRTI have the potential to cause lung damage that may lead to temporary or permanent alterations in lung function. Several retrospective studies have suggested that a documented LRTI in infancy and childhood increases the risk of chronic respiratory symptoms and reduced lung function in later childhood and adulthood.[10 11 177-181] Viral respiratory infections, particularly rhinovirus, in early life are associated with the later development of asthma.[182 183] However, none of these studies included baseline lung function measures and hence these studies were unable to distinguish between the respiratory infections or pre-existing respiratory disease vulnerability as the cause of decreased lung function or chronic respiratory disease. Two groups have prospectively assessed the impact of early life respiratory tract infections on later lung function. Castro-Rodriguez et al investigated 133 children longitudinally with lung function at two months and again at 11 years, collecting information on inter-current respiratory tract infections, both those with and without radiological pneumonia.[184] In this study children with respiratory tract infections without radiologically defined pneumonia had reduced premorbid lung function at two months, which persisted to 11 years. Children with radiologically confirmed pneumonia have reduced lung function at 11 years, but only eight of these children had baseline lung function measured and hence they were not able to accurately assess the role baseline lung function played in risk for pneumonia or subsequent lung function deficit.[184] The Oslo birth cohort, Environment and Childhood Asthma study, prospectively followed a cohort of 607 infants with lung function at birth, two years and ten years of age, recording LRTI during the first two years of life.[78] This

study suggests that LRTI in the first two years of life do not impact on normal lung growth between birth and ten years of age, nor the lung function achieved at ten years. The LRTI incidence in this cohort during the first two years was 22% and the majority of infections (56%) were bronchiolitis episodes.

**5. Determinants of lung function in the African context**

There have been no studies investigating lung function growth in African children. Given the importance of early lung health on subsequent lung disease there is an urgent need for research on lung function of African infants.

Several factors are important to the African context including HIV exposure, indoor air pollution and early life LRTI. HIV infection is well known to cause acute and chronic respiratory disease.[185] Exposure, but not infection, to HIV in utero could affect lung growth through the direct effects of the HIV virus, consequences of immune suppression or exposure to antiretroviral therapy. Prevention of mother to child transmission of HIV infection includes antiretroviral therapy (ART) taken by the mother throughout pregnancy. There are no published data comparing lung function in HIV exposed uninfected and HIV unexposed infants. Exposure to in utero ART in HIV exposed uninfected infants is associated with anaemia, neutropenia, thrombocytopenia, mitochondrial abnormalities and cardiac function effects (increased fractional shortening and decreased left ventricular mass).[186 187] It is possible exposure to HIV in utero may impact lung function and growth and impact respiratory health in exposed infants. The impact of early life LRTI on normal lung growth and subsequent lung function is also not clear. Given the high incidence of childhood LRTI and chronic respiratory disease in Africa, this is important to investigate.

Exposure to air pollutants is likely to be very different to that of the infant lung function cohorts already studied. Africa has a high rate of biomass fuel use.[188] Overcrowding and high density living increase potential for high exposure to air pollution and cigarette smoking is increasing in low income households.[189] Understanding the contribution of local environmental exposures on lung health is important.

In summary many factors known to impact early lung function and increase risk of repsiratory disease are common in Africa. Better understanding the impact of these factors

in African infants will improve our ability to develop appropriate strategies to prevent or ameliorate damage to lung growth and reduce risk of respiratory disease.

## Aims

The overall aim of this thesis is to describe lung function in South African infants from six weeks to one year and to investigate the impact of prenatal and early environmental exposures on lung function in infancy.

### Specific aims:

1. To describe lung function in African infants and to develop normative data ranges at six weeks of age for this population.
2. To investigate the impact of antenatal and early life exposures on lung function at six weeks of age.
3. To longitudinally measure lung function at six weeks and one year in South African infants.
4. To investigate the impact of nutritional, immunological and environmental factors; infant lung function and intercurrent lower respiratory tract infections during the first year of life on lung function outcomes at one year of age.

### Hypotheses

1. Reduced infant lung function (six weeks of age) is associated with reduced lung function at one year.
2. Antenatal and early post-natal exposures including *nutritional factors* (poor growth and/or obesity), *immunological factors (*atopy, or acquired immune deficiency), *environmental factors* (household air pollution, including smoking) and/or *infective factors (*early postnatal respiratory infections) alter lung structure in utero and/or in early life and are associated with altered lung function outcomes in early infancy (six weeks of age and at one year of age)
3. Lung function tracks from six weeks to one year of age.

**The chapters of the thesis will address the following:**

1. Establishing infant lung function testing in a South African peri-urban setting: description of study methodology.
2. Assessing the feasibility of a novel measure of infant respiratory impedance.
3. Describing lung function in African children; and development of normative data ranges at six weeks of age for this population.
4. Investigating the impact of antenatal and early life determinants on early infant lung function (six weeks of age).
5. Longitudinal investigation of lung function from six weeks to one year of age and investigating the impact of nutritional, immunological and environmental factors; infant lung function and intercurrent respiratory tract infections on lung function at one year.

**Table 1: Infant lung function tests**

| | Plethysmography | Multiple breath washout | Tidal breathing flow volume loops | Spirometry: RTC and RVRTC | Single breath occlusion technique | Interrupter technique | Forced oscillation technique | Exhaled Nitric oxide |
|---|---|---|---|---|---|---|---|---|
| **Outcome measured** | Lung volumes: $FRC_{pleth}$ Lung mechanics: $R_{aw,}$ $G_{aw}$ | Lung volumes: $FRC_{MBW}$ Ventilation homogeneity: LCI | Lung volumes: $V_T$ Respiratory rate Air flow: $t_{PTEF}/t_E$, MIF, MEF | Forced expiratory volumes: FVC, $FEV_t$ Air flow: $V_{max}FRC$, $FEF_{\%FVC}$ | Mechanics: $C_{rs}$, $R_{rs}$, Time constant | Lung mechanics: $R_{int}$ | Lung mechanics: $R_8$, $X_8$, $C_{rs}$, $R_{rs}$, Resonance frequency | Airway inflammation: Nitric oxide |
| **Standardised equipment** | Yes | Yes | Yes | Yes | Yes | Yes | No | Yes |
| **Published testing standards** | Yes | Yes | Yes | Yes | Yes | Yes | No | Yes |
| **Adequate reference data for African infants** | No | No | No | No | No | No | No | No |
| **Tidal breathing measure** | Yes | Yes | Yes | No | No | No | Yes | Yes |
| **Sedation required** | Yes | No | No | Yes | No | No | No | No |
| **Used in epidemiological studies** | Yes | Yes | Yes | Yes | Yes | Yes | Yes | Yes |

RTC: rapid thoracic compression; RVRTC: raised volume rapid thoracic compression; $FRC_{pleth}$: plethysmographic functional residual capacity; $R_{aw}$: airway resistance $G_{aw}$: airway conductance $FRC_{MBW}$: multiple breath washout functional residual capacity; LCI: lung clearance index; $V_T$: tidal volume; $t_{PTEF}/t_E$ : ratio of time to peak expiratory flow over total expiratory time; MIF: mean inspiratory flow; MEF: mean expiratory flow; FVC: forced vital capacity; $FEV_t$: forced expiratory volume in unit time; $V_{max}FRC$: maximal flow at FRC; $FEF_{\%FVC}$ : forced expiratory flow at % FVC; $R_{rs}$: respiratory system resistance; $C_{rs}$ respiratory system compliance

**Table 2: Birth cohort studies with longitudinal lung function measured in early life**

| Cohort | Tuscon[20] 23 84 159 190 191 | Perth[21 85] 135 160 192-194 | WAPC-Raine 76 | Boston[86] 158 195 196 | Hammersmith 27 32 68 | London[29 33] 59 60 77 139 197 | Manchester 147 198 199 | Copenhagen 87 88 | Southhampton 30 49 | Oslo[55 74 200] | Bern [38 56] 121 201 | Utrecht[24 93] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Birth years** | 1980-1984 | 1987-1991 | NS, over 1 year | 1988-1992 | ns | NS, over 5 year | ns | 1998-2001 | 1999-2002 | 1992-1993 | 1999 -2010 | 2001- |
| **Inclusion criteria** | Healthy; enroled at birth | Healthy, term infants; enroled at birth | | Mother >19yrs; early booking, healthy | Healthy, term, one atopic parent | Caucasian, term, healthy | | Mother: doctor diagnosed asthma; term, healthy | Term, healthy; enroled at birth | Healthy, birthweight >2 kg | Term, healthy, caucasian | Term, healthy; enroled at birth |
| **Number enrolled** | 1246 | 253 | 500 | 1000 | unclear | 118 | 69 | 411 | 147 | 803 | 342 | 450 |
| **Age lung function** | 8 wks | 3-4 wks | 2.4 (1-7) days | 2-6 wks | 2-4 wks | 1-3 mths | 4-6 weeks | 1 month | 5-14 weeks | birth | 5 weeks | 3-8 weeks |
| **Lung function test** | RTC; He-MBW; TBFVL; FOT | RTC; histamine; TBFVL, SOT | Inductance plethysmog-raphy: respirtory rate, $t_E$, $t_{PTEF}/t_E$ | VmaxFRC; He-MBW; TBFVL; SOT | RTC, BHR-histamine challenge, TBFVL, SOT | Plethmog-raphy (sGaw), MOT, TBFVL | RTC | RVRTC, BHR - metacholine, eNO | RTC, TBFVL | TBFVL, SOT (unsedated) | TBFVL, SF6-MBW, Rint, eNO (unsedated) | SOT (unsedated) |
| **Number tested (% enrolled)** | 124 (10%) | 252 (99%) | 461 (92%) | 159 (32%) | 73 | 108 (92%) | 69 (100%) | RVRTC: 403 (98%), BHR 362 (88%) | 147 (100%) | 802 (99%) | 342 (100%) | 328 (73%) |
| **Age repeat lung function** | 6, 11, 18, 22 years | 6mth, 1 year, 6 years | 6 years, 14 years | 4-6, 9-12, 15-18 month | 6 and 12 months | 1 year | 3, 5, 8-9 years | 7th year | none but skin prick test 3 year | 10 and 16 years | | 5th year |
| **Lung function test** | Spirometry | RTC (6 mth and 1 yr) Spirometry (6 yr) | Spirometry | VmaxFRC; He-MBW; TBFVL; SOT | RTC, BHR-histamine challenge, TBFVL, SOT | Plethys-mography (sGAW), MOT, TBFVL | Plethys-mography (3 and 5 yr), spirometry, BHR-meta-choline (8-9 yr) | Spirometry | NA | Spirometry | NA | Spirometry |

*WAPC: Western Australian Pregnancy Cohort (Raine) Study; VmaxFRC: maximal flow at functional residual capacity; He-MBW: helium multiple breath washout; TBFVL: tidal breathing; FOT: forced oscillation technique; SOT: single breath occlusion technique; $t_E$: total expiratory time; $t_{PTEF}/t_E$: time to peak tidal expiratory flow over total expiratory time; sGaw: specific airway conductance; BHR: bronchial hyper-responsiveness testing; RTC: rapid thoracic compression; RVRTC: raised volume rapid thoracic compression; eNO: exhaled nitric oxide; Rint: Interrupter technique resistance; SF6-MBW: sulphur-hexafluoride multiple breath washout

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Chapter 2

# Lung function in African infants: a pilot study.

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## Abstract

**Background:** The burden of childhood respiratory illness is large in low and middle income countries. Infant lung function testing may provide useful information about lung growth and susceptibility to respiratory disease. However, ILF has not been widely available in low and middle income settings where the greatest burden of childhood respiratory disease occurs.

**Aim:** To implement and evaluate a pilot study of infant lung function testing in a semi-rural setting in South Africa.

**Method:** Infant lung function testing was established at a community hospital in South Africa. All measures were done in unsedated infants during sleep. Measurements, made with the infant quietly breathing through a face mask and bacterial filter, included tidal breathing (TBFVL), exhaled nitric oxide (eNO) and sulphur hexafluoride multiple breath washout (MBW) measures using an ultrasonic flow meter and chemoluminescent NO analyzer.

**Results**: Twenty infants, mean age of 7.7 (SD 2.9) weeks were tested; 8 (40%) were Black African and 12 (60%) were mixed race. Five (25%) infants were preterm. There were 19 (95%) successful TBFVL and NO tests and 18 (90%) successful MBW tests. The mean tidal volume was 30.5 ml (SD 5.9), respiratory rate 50.2 breaths per minute (SD 8.7) and eNO 10.4 ppb (SD 7.3). The mean MBW measures were: functional residual capacity 71 ml (SD 13) and the lung clearance index 7.6 (SD 0.5). The intra-subject coefficients of variations of lung function measures were similar to published normative data for Caucasian European infants.

**Conclusion:** In this study we demonstrate that unsedated infant lung function measures of tidal breathing, MBW and eNO are feasible in a semi-rural African setting with rates comparable to those reported from high income countries.

**Keywords**

Respiratory function test, South Africa, paediatric

## Background

Lung development is incomplete at birth with much lung growth occurring during the first few years of life. Assessment of lung function longitudinally from birth may offer valuable information about the true burden, determinants and outcomes of low lung function in early life. An objective measure of lung function is necessary to achieve this. Until recently infant lung function (ILF) testing has been largely restricted to isolated research institutions using custom made equipment. This made it difficult to compare results reducing generalizability among sites. Equipment was often bulky, testing moderately invasive and almost exclusively done under sedation, which limited its use in well infants and particularly its use in community settings. Recent advances in technology, standardisation of ILF measurement equipment and techniques through international expert collaboration has allowed the development of tests that are easily done in unsedated infants and have made ILF a more accessible and useful tool.[1 2] ILF is now routinely used in high income countries as an epidemiological and clinical tool.[3-6]

The burden of acute and chronic respiratory disease is high in low and middle income countries (LMICs). Respiratory illness is a leading cause of early childhood death in LMICs.[7 8] In addition chronic respiratory illness is increasingly common and associated with a substantial burden of disease in African children and indeed globally.[9] Developing respiratory function measures that can be used in these settings to assess determinants of respiratory disease is desirable.

However little is known about ILF in African children, a region with one of the highest burdens of childhood respiratory disease.[9 10] Infant lung function has not been previously undertaken in South Africa or any other African setting. We hypothesised that unsedated infant lung function measures, including tidal breathing, exhaled nitric oxide and multiple breath washout testing, can be safely and successfully undertaken in a semi-rural setting in the Western Cape, South Africa. Hence we aimed to demonstrate feasibility of ILF in this setting and to provide pilot data for a larger birth cohort study, the Drakenstein Child Lung Health Study (DCLHS).

## Method:

### Ethics

The study was approved by the Faculty of Health Sciences, Human Research Ethics Committee, University of Cape Town (401/2009) and by the Western Cape Provincial Health Research committee. Written informed parental consent was obtained in the first language of the parent.

### Site Description

Infant lung function testing was established at Paarl hospital, a community hospital located in the semi-rural Drakenstein region. The hospital serves a low socio-economic community of approximately 200 000 people; all births in the public sector (approximately 90% of all births in the area) occur at this hospital.

### Participants

Infants born between March and June 2012 at Paarl hospital, South Africa were eligible for enrolment. Infants born during enrolment period, who were healthy and whose parents gave consent were eligible for inclusion. Any infant with an acute or chronic illness was not included. Infant testing was booked for 6 to 10 weeks of age and was delayed until at least 2 weeks after any acute respiratory infection.

### Infrastructure development and training

An international collaboration was established between paediatric pulmonologists in South Africa and ILF specialists in Australia. The local investigator and respiratory technologist were trained in Perth, Australia during Nov-Dec 2011 in ILF testing techniques. Training included the set up, calibration, data collection and analysis of the tidal breathing measures (TBFVL), exhaled nitric oxide (eNO) and multiple breath washout (MBW) techniques. A second technologist was trained on-site during the set up period. Standard operating procedures were established with oversight from the international collaborators, who also provided on-going supervision and training through regular teleconference. During this period early studies were over-read by the experts in Perth with feedback to the study team. The equipment used was the EcoMedics ExhalyzerD with ultrasonic flow

meter and CLD88 NO chemoluminescent analyser (EcoMedics, Duernten, Switzerland). The company provided an initial introduction to the equipment during the set up; the local team was then assisted through email and teleconference communication during the first months for troubleshooting. Supply chains for consumables including SF6 testing and NO calibration gas mixtures were successfully established.

**Infant Lung Function**

Infant lung function was tested between 1 to 3 months of age. All tests were done by the same testing team, which consisted of a trained respiratory technologist and a professional nurse, supervised by a paediatric pulmonologist. Testing was measured in unsedated infants during behaviourally defined quiet natural sleep.[11] If the child woke during a measurement the child was settled and testing restarted when the child was in quiet sleep. Measurements were taken with infants lying supine with their head in the midline using a size 0 round infant silicone placed over the nose and mouth. The effective dead space of the mask (5mL) was determined as the water displacement volume of the mask on a curved surfaced and applying similar pressure to the mask as used during an actual test. The water displacement volume was then halved to determine the effective mask deadspace as recommended previously [1 12] and as published by our group.[13 14] Testing was performed on commercially available equipment that conformed to the ATS/ERS ILF equipment standards.[1] Flow was measured using a commercially available ultrasonic flow meter (Ecomedics AG, Duernten Switzerland). A dead space reducer (size 1) was used to reduce the internal dead space of the flow meter to 1.2 ml. A disposable bacterial filter (SpiretteTM, EcoMedics AG, Duernten, Switzerland) surrounded the dead space reducer. A bias flow of 200 $mL \cdot s^{-1}$ was used. During data collection, flow-volume loops were observed to ensure there was no leak. Data were recorded at ambient temperature, pressure and saturation with flow and volume being converted to body temperature and pressure, saturated conditions during data analysis. Leak was defined as volume drift of $>3ml.s^{-1}$ and data excluded in these cases. Measurements were collected in the following order: TBFVL and simultaneous eNO for 90 seconds, followed by three acceptable MBW measurements where possible. Data analysis, detailed for each test below, was independently repeated by experts in Perth to assure quality control. Test analysis was

blinded with neither of the two sites, Perth and Drakenstein, having reference to the other score.

**Tidal breathing recordings (TBFVL)** were obtained during quiet tidal breathing at least 30 seconds after initial mask placement. Recordings were analysed if >30 consecutive regular breaths of tidal breathing were recorded during a 90 second epoch and according to international guidelines.[15] Mean tidal breathing measures were calculated using analysis software (Wbreath v3.2.0, Ndd Medizintechnik AG, Zurich, Switzerland) as reported previously.[16 17] Outcome parameters included respiratory rate, tidal volume, minute ventilation, mean tidal inspiratory flow, mean tidal expiratory flow and the ratio of time to peak tidal expiratory flow /expiratory time as a description of the shape of the tidal breathing flow volume loop.

**Exhaled nitric oxide (eNO)** measurements were recorded simultaneously with the TBFVL and according to international guidelines and as previously published by our group.[17 18] Exhaled NO was an online measurement with a rapid-response, chemoluminescence analyser (CLD 88 Exhalyzer, Ecomedics AG, Duernten, Switzerland). Testing used NO free air for inspiration avoiding contamination from ambient NO. Exhaled NO was calculated breath by breath during the third quartile of expiration and the mean eNO calculated from a minimum of 30 recorded breaths using the analysis software (Wbreath v3.2.0, Ndd Medizintechnik AG, Zurich, Switzerland). The outcome parameters recorded included the eNO and the NO output (eNO x corresponding expiratory flow).

*Multiple breath washout (MBW)* testing was performed using 4% SF6 as a tracer gas and ultrasonic flow meter (Spiroson©, Ecomedics AG, Duernten, Switzerland) with acquisition and analysis software (Wbreath v3.2.0, Ndd Medizintechnik AG, Zurich, Switzerland) as reported by our group [13] and in accordance with recent recommendation for the measurement of MBW.[19] The washout period began after a ten breath equilibrium period was obtained at the end of the tracer gas wash in. The washout continued until the tracer gas was eliminated from the lungs. The outcome parameters included the functional residual capacity (FRC) and the lung clearance index (LCI; cumulative expired volume/ FRC) and moment ratios (M1/M0 and M2/M0). The recordings were defined as accept-

able for analysis if they occurred during quiet sleep with no sighs within 10 breaths of the wash-in plateau or 10 breaths after the SF6 concentration has returned to baseline. The process was repeated to obtain three successful recordings. If the child woke testing was restarted when infant went back to sleep, provided the parent was happy to wait. Analysis of the recordings using the analysis software (Wbreath v3.2.0, Ndd Medizintechnik AG, Zurich, Switzerland), included the use of optimised temperature and deadspace model.16 Flow and volume were converted to body temperature and pressure, saturated conditions during data analysis. The mean FRC and LCI value of the 3 tests was reported or the mean of 2 tests if the mean was within 10% of the lower value.

**Statistical analysis**

Descriptive statistics were performed using STATA 11 for windows (STATA Corporation, College Station, USA). Data are presented as mean and standard deviation (SD), median and interquartile range (IQR) and the 95% limits of agreement, which was defined as the mean difference ± 1.96 SD. The intra-subject coefficient of variation (CV) was calculated as the ratio of each parameter standard deviation over the parameters mean per study participant. For FRC and LCI this was calculated from the mean and standard deviation of the MBW tests collected. The agreement between observers' results from the two sites was presented as the mean difference and SD, and minimum and maximum values.

**Results**

The demographics of included infants are summarised in **Table 1**.Twenty infants with a mean age of 7.7 (range 3.4 to 14) weeks consented for participation. Five (25%) were preterm, defined as born before 37 weeks post gestational age. Of the infants tested one TBFVL and one eNO test was excluded due to poor quality trace on analysis (less than 30 regular breaths selected), thus there were 19 (95%) successful TBFVL and eNO tests. Two MBW tests were excluded as one child woke up and could not complete the test and in the second the mean of the two tests obtained was not within 10% of the lower value; there were therefore 18 (90%) successful MBW tests. The median time taken per infant test was 22 min (range 6 to 64). Six (30%) infants woke during testing and had to be settled for a second measurement. One infant did not sleep and required rebooking

on another day to complete testing. The median length of lung function visit, including waiting time for infants to sleep was 159 min (range: 27 to 320).

Tidal breathing and MBW results are shown in **Table 2**. The mean (SD) difference between the blinded analyses of the Perth and Drakenstein sites for FRC in mL was 0.17 (0.5), range: 0 to 2.2 and LCI 0.05 (0.1); range: 0 to 0.5. The intra-subject coefficient of variation (CV) demonstrates the variability for each outcome and ranges from 6.5% for minute ventilation (V'E) to 19.7% for the ratio of time to peak tidal expiratory flow over the total expiratory time ($t_{PTEF}/t_E$).

The multiple breath washout test results, **table 2**, include measures of lung volume, the functional residual capacity (FRC) and measures of ventilation inhomogeneity the lung clearance index (LCI) and moment ratios. The intra-subject CV was 6.8% for the FRC and 3.6% for the LCI. The exhaled nitric oxide results, **table 2**, include exhaled NO and NO output.

**Discussion**

This study describes the successful implementation of an ILF testing site in a semi-rural setting in South Africa. This is the first time ILF testing, predominantly used in specialised centres in high income settings, has been used in Africa.

The success rates for testing were similar to those reported by Fuch's et al[20], who recorded an 85% testing success for TBFVL measures, 76% for eNO and 59% for MBW testing in 342 healthy unsedated European infants at 4 to 9 weeks of age. Although the equipment and testing procedures used in this study were similar, the Fuchs study used a longer tidal breathing epoch of 100, compared to 30, consecutive regular breaths during quiet sleep. This would have prolonged testing and possibly reduced the chance of successfully recording all measures before infants woke.

Successful implementation depended on several key factors. These included close and ongoing collaboration with international experts experienced in ILF testing, initial training in an experienced laboratory, back up technical support from the supplier of the equipment, sufficient resources for equipment, training and staffing, a defined research space

and dedicated, well trained staff onsite. The initial training of a local technologist in the overseas ILF centre allowed for the training of further local technologists. This collaboration ensured strict supervision of data quality and allowed double analysis of data. This collaborative work is possible with standard internet connectivity and did not necessarily require teleconference facility.

The median time of testing during this study was 22 minutes (IQR 6; 64). This only included active testing time. Six (30%) of the infants required resettling and a second attempt at testing hence the total visit time [median (range): 159 min (27; 320)] was long in these infants. Thus successful completion of unsedated infant lung function testing can be time consuming. Importantly, no sedation was used, thus the measurements reflect physiological lung volumes during sleep. Furthermore, this makes the procedure especially safe and reduced the need for post test observation and monitoring.

The results of the testing were comparable to the normative values for European infants published by Fuchs et al (**Table 3**) [20]. This comparison is however limited by the pilot studies small sample size, and should be confirmed in a larger cohort from a similar South African community. In addition the pilot cohort included infants born prematurely, a known risk factor for early low lung function.[21] However none of our infants were born less than 33 weeks gestation and mean gestation was 37 weeks. Twenty five percent of infants' mothers smoked during pregnancy. Maternal smoking is a known risk factor for low lung function at birth.[22 23] The results of this study cannot be used to inform normative ranges for African children given the small sample size of this pilot and the inclusion of infants at risk of low lung function.

The results had acceptable variability, as expressed by the intra-subject CV, supporting the feasibility of the proposed larger project. In the tidal breathing measures the CV for each parameter was less than 10% except for the $t_{PTEF}/t_E$, which was 19.7%. This is consistent with previously published results of similar testing in unsedated infants.[20 24] The mean FRC in this study 70.7 (SD 13) was lower than the European study, 102 (SD 16). The LCI was higher, 7.6 (SD 0.5) compared to 6.7 (SD 0.6) in the European study. The infants in the South African cohort were an average of 2 weeks older than the European

cohort. The difference in FRC and LCI may relate to the different ethnicity or socio-economic status of this group, factors that have been previously reported to effect lung function measurements in young children.[25 26] A larger cohort is needed to confirm this and investigate possible associations. The mean eNO in this study of 10.4 (SD7.3) was lower than the 14.3 (SD 6) reported by Fuchs et al. However the sample size is too small to make meaningful comparisons and further study with a larger sample is required.

In conclusion, this pilot study has described the development of technical and clinical capacity in ILF testing in a LMIC setting. This study provides reliable data for supporting the use of these measures in a prospective birth cohort study, the Drakenstein Child Lung Health Study. This technique has the potential to be a useful tool in obtaining valuable information on early lung growth, function and the development of acute and chronic lung diseases in children living in high burden settings including Africa.

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**Table 1 Demographics of infants (n=20)**

| Characteristic at study date | Mean (SD) | Med (min; max) |
|---|---|---|
| Age (weeks)[1] | 7.7 (2.9) | 6.6 (3.4; 14) |
| Weight (kg) | 4.6 (0.99) | 4.5 (3; 6.2) |
| Height (cm) | 51.5 (3.1) | 50.5 (47; 57) |
| Time taken for testing (min) | 23 (12) | 22 (6; 64) |
| Gestational age at birth (weeks) | 37 (1.8) | 37 (33; 39) |
| | N (%) | |
| Maternal smoking in pregnancy | 5 (25) | |
| Female gender | 13 (65) | |
| Ethnicity | | |
| Black African | 8 (40) | |
| Mixed race | 12 (60) | |

[1] Age corrected to 37 week gestation for preterm infants

**Table 2 Tidal breathing, multiple breath washout and exhaled nitric oxide measurements**

| Tidal breathing | Number n | Mean (SD) | Median (IQR) | 95% limits of agreement | Median (IQR) intra-subject CV[4] |
|---|---|---|---|---|---|
| Tidal volume ml | 19 | 30.5 (5.8) | 30.2 (25.3; 34.4) | 18.9 - 42.1 | 6.8 (6; 7.9) |
| Respiratory rate breaths per min | 19 | 49.4 (8.4) | 49.6 (42.9; 54.8) | 41 - 65.9 | 7.7 (6.5; 9.3) |
| Minute ventilation ml/min | 19 | 1487.4 (319.8) | 1433.5 (1275.3; 1634.7) | 860.6 –2114.2 | 6.5 (4.6; 8.3) |
| $t_{PTEF}/t_E$[1] | 19 | 37 (11.5) | 35 (27; 44.8) | 7.46 – 34.26 | 19.7 (15.6; 25.2) |
| MTIF[2] | 19 | 54.8 (10.2) | 54.3 (50; 57.3) | 34.9 – 74.7 | 6.6 (5.4; 10) |
| MTEF[3] | 19 | 46 (12) | 45.8 (36.2; 52) | 22.3 – 69.7 | 7.8 (6.2; 10) |
| **Multiple breath washout** | **Number n** | **Mean (SD)** | **Median (IQR)** | **95% limits of agreement** | **Median (IQR) intra-subject CV[4]** |
| FRC[5] ml | 18 | 70.7 (13) | 66.3 (61; 83) | 45.2 - 96.2 | 6.8 (4.5; 8.8) |
| LCI[6] | 18 | 7.6 (0.5) | 7.5 (7.3; 7.8) | 6.6 – 8.6 | 3.6 (1.6; 6.4) |
| Moment ratio m1/m0 | 18 | 1.5 (0.1) | 1.5 (1.3; 1.6) | 1.3- 1.7 | |
| Moment ratio m2/m0 | 18 | 5.6 (0.8) | 5.8 (4.2; 6.6) | 4 -7.2 | |
| **Exhaled nitric oxide** | **Number n** | **Mean (SD)** | **Median (IQR)** | **95% limits of agreement** | **Median (IQR) intra-subject CV[4]** |
| NO ppb | 19 | 10.4 (7.3) | 9.7 (3.4; 17.2) | -4 – 24.7 | 4.2 (3.3; 5.5) |
| NO output mcl/s | 19 | 7.8 (2.2) | 7.2 (6.2; 9.1) | 3.5 – 12.1 | 2.3 (0.8;3) |

[1]Ratio of time to peak tidal expiratory flow over total expiratory time [2]Mean tidal inspiratory flow [3]Mean tidal expiratory flow [4]Coefficient of variation, [5]Functional residual capacity [6]Lung clearance index

**Table 3 Drakenstein infant lung function compared to European birth cohort**

| | **Drakenstein Pilot Study N=20** | | **European study N=296 20** | |
|---|---|---|---|---|
| | Mean (SD) | Median (IQR) intra-subject CV5 | Mean (SD) | Median (IQR) intra-subject CV5 |
| **Tidal breathing N successful test (%)** | **19 (95)** | | **285 (96)** | |
| Tidal volume ml, mean (SD) | 30.5 (5.8) | 6.8 (6; 7.9) | 32.4 (5.5) | 8.6 (7.1; 10.8) |
| Respiratory rate breaths per min, mean (SD) | 49.4 (8.4) | 7.7 (6.5; 9.3) | 45.2 (10.5) | 9.1 (7.4; 11.3) |
| Minute ventilation ml/min, mean (SD) | 1487.4 (319.8) | 6.5 (4.6; 8.3) | 1420 (277) | 7.5 (6.1; 10.1) |
| $P_{TEF}/T_E$[1] | 37 (11.5) | 19.7 (15.6; 25.2) | 34.8 (10.7) | 23.8 (20.2;28.4) |
| **Multiple breath washout N successful tests (%)** | **18 (90)** | | **201 (68)** | |
| FRC[2] ml, mean(SD) | 70.7 (13) | 6.8 (4.5; 8.8) | 102 (16) | 6.3 (4.4; 8.3) |
| LCI[3] | 7.6 (0.5) | 3.6 (1.6; 6.4) | 6.75 (0.6) | 5.8 (3.6; 8.0) |
| **Exhaled nitric oxide N successful tests (%)** | **19 (95)** | | **261 (88)** | |
| NO[4] ppb | 10.4 (7.3) | 4.2 (3.3; 5.5) | 14.3 (6) | 10.3 (6.4; 15.7) |
| NO output mcl/s | 7.8 (2.2) | 2.3 (0.8;3) | 6.3 (2.5) | 12.4 (8.9–18.1) |

[1]Ratio of time to peak tidal expiratory flow over total expiratory time, [2]Functional residual capacity, [3]Lung clearance index, [4]Nitric oxide, [5]Coefficient of variation

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Chapter 3

# Respiratory Impedance in Healthy Unsedated South-African Infants: effects of maternal smoking

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## Abstract

**Background:** Non-invasive techniques for measuring lung mechanics in infants are needed for a better understanding of lung growth and function and to study the effects of prenatal factors on subsequent lung growth in healthy infants. The forced oscillation technique requires minimal co-operation from the individual, but has rarely been used in infants.

**Aim:** To assess the use of the forced oscillation technique to measure the influence of antenatal exposures on respiratory mechanics in unsedated infants enrolled in a birth cohort study in Cape Town, South Africa.

**Method:** Healthy term infants were studied at 6-10 weeks of age using the forced oscillation technique. Respiratory impedance was measured in the frequency range 8-48 Hz via a facemask during natural sleep. Respiratory system resistance, compliance and inertance were calculated from the impedance spectra.

**Results:** Of 177 infants tested, successful measurements were obtained in 164 (93%). Median (25-75%) values for resistance, compliance and inertance were 50.2 (39.5-60.6) $cmH_2O.s.L^{-1}$, 0.78 (0.61-0.99) $mL.cmH_2O^{-1}$ and 0.062 (0.050-0.086) $cmH_2O.s^2.L^{-1}$, respectively. As a group, male infants had 16% higher resistance (p=0.006) and 18% lower compliance (p=0.02) than females. Infants whose mothers smoked during pregnancy had a 19% lower compliance than infants not exposed to tobacco smoke during pregnancy (p=0.005). Neither maternal HIV infection nor ethnicity had a significant effect on respiratory mechanics.

**Conclusions:** The forced oscillation technique is sensitive enough to demonstrate the effects of tobacco smoke exposure and sex in respiratory mechanics in healthy infants. This technique will facilitate assessing perinatal influences of lung function in infancy.

**Keywords**: respiratory function test, forced oscillation technique, respiratory compliance, respiratory resistance, paediatric

## Introduction

The assessment of lung mechanics in healthy infants offers the potential to better understand normal lung growth and function, the determinants of early lung development and the relationship between lung function and respiratory disease. The forced oscillation technique (FOT) is a promising tool for measurement of lung function in infants as it is non-invasive, versatile and does not require controlled respiratory manoeuvres.[1] Moreover, the small-amplitude oscillations are superimposed on spontaneous breathing, so measurements can be taken without interfering with normal respiration.

As a consequence of the non-invasive nature and the minimal demand for co-operation the FOT has gained popularity in paediatric lung function testing, and several coherent normative datasets have been published.[2 3] However, its use in infancy has been sporadic and largely confined to methodological validation studies,[4-13] all using sedation except one.[11] The development of a FOT approach that is able to non-invasively measure respiratory system impedance (Zrs) in infants during natural sleep would provide the opportunity to track the mechanical properties of the lung through the early years of life, a time of critical lung growth and development. In addition to the establishment of normative data in infancy, such a method would be useful in the studies on effects of prenatal factors that may impact on later respiratory health, such as maternal smoking and HIV infection, the prevalence of which is high in South African populations.

The purpose of this study was therefore to (i) describe the mechanics of the respiratory system using FOT in healthy unsedated infants and (ii) assess the impact of antenatal and early life factors on respiratory mechanics in infants from a large birth cohort study in a low- middle-income setting in South Africa.

## Methods

Healthy infants aged 6 to 10 weeks enrolled in the Drakenstein Child Lung Health study, a birth cohort study established in a peri-urban area outside Cape Town, South Africa, were included in the study. The study population are low socioeconomic of African ancestry, enrolled from health clinics serving two predominantly low socio-economic commu-

nities. Details of the study population and setting are already published [14] and described in the online supporting information. The study was approved by the Faculty of Health Sciences, Human Research Ethics Committee, University of Cape Town (401/2009) and by the Western Cape Provincial Health Research Committee. Mothers gave informed, written consent in their first language for their infants to participate.

**Measurement of lung function**

Zrs was measured with purpose-built FOT equipment (Fig. 1). A composite driving signal (frequencies: every 4 Hz between 8 and 48 Hz, pressure amplitude: <1 $cmH_2O$) was generated by a loudspeaker and delivered to the infant through a wave-tube (internal diameter: 1 cm), an anti-bacterial filter (Humid-Vent, no. 19502, Teleflex Medical) and a facemask (Neonate crystal anaesthesia mask, no. 39170, Koo Asia, Hong Kong). The inlet and outlet pressures of the wave-tube were sensed by ICS transducers (Model 33NA002D ICSensors, Miltipas, CA). Zrs was calculated as the load impedance on the tube,[15] after corrections for the equipment impedances of the filter and the facemask. A pneumotachograph with a differential pressure transducer (ICS model 33NA002D) was attached to the wave-tube for monitoring the infant's breathing pattern. The dead space of the equipment was continuously flushed by a bias flow of air at 2 $L.min^{-1}$.

The measurements of Zrs were made during quiet sleep in the supine position, with the head supported in a neutral position. A minimum of 5 technically acceptable 30-s data epochs were collected. Recordings (or short segments of them) that contained breath holds, cries, irregular breathing or leaks around the facemask were excluded. Zrs spectra were defined as reproducible if at least 3 of the spectra had Zrs magnitude values within 10% of each other. The individual Zrs spectra were evaluated by fitting a resistance (R) – compliance (C) - inertance (I) model to the measured data and the results averaged (see the online supporting information for details). Resonance frequency (fres) was calculated as $fres=1/(2\pi\sqrt{CI})$.

**Collection of antenatal and early life data**

Information regarding antenatal, birth and early life exposures and events were collected by questionnaires at scheduled study visits.[16] Maternal smoking was confirmed by a

quantitative analysis of maternal urine cotinine, and all mothers underwent HIV testing at an antenatal visit and at birth; details of these tests are described in the online supporting information.

### Statistical analysis

Statistical analysis was performed using STATA 13 (STATA Corporation, College Station, Texas, USA). Data are presented as mean, standard deviation (SD), median and 25-75% and 95% confidence intervals. The intra-subject coefficient of variation (CoV) was calculated as CoV=100SD/mean for each infant's measurement. The relationships between anthropometric variables, prenatal/perinatal data and respiratory mechanics were examined using the Wilcoxon rank-sum test for independent samples and Spearman's correlation analysis. A multivariate analysis examining the determinants of the Zrs parameters was undertaken, as detailed in the online supporting information. Differences at a p value of $<0.05$ were considered statistically significant.

### Results

Of 219 infants tested, 42 infants who were born preterm or had previous pneumonia were excluded leaving 177 healthy infants; 164 (93%) of whom had acceptable data collected (see Fig. S1 in the online supporting information for exclusion categories). The demographic data of the 177 infants are shown in Table 1.

The values of impedance parameters R, C, I and *f*res are summarized in Table 2. The intra-subject variability, represented by the median (25-75%) of the intra-individual CoV is also included. The Zrs parameters showed strong interdependences (see Fig. S2 in the online supporting information) with a negative correlation between C and R ($r=-0.52$; $p<0.001$), and positive correlation between I and R ($r=0.57$; $p<0.001$).

The association of demographic factors and early life exposure and respiratory mechanical parameters measured with FOT are represented in Fig. 1. Male infants had 10% higher R (53.2 [40.9-65.4] vs. 48.3 [37.2-58.8] cmH2O.s.L-1, $p=0.005$) and 17% lower C compared to female infants (0.72 [0.53-0.9] vs. 0.87 [0.69-1.08] mL.cmH2O-1, $p=0.004$); I and fres were similar in male and female infants. Infants whose mothers

smoked during pregnancy had a 21% lower C (0.71 [0.54-0.96] vs. 0.92 [0.80-1.20] mL.cmH2O-1, p=0.005) and 19% higher fres (24.6 [20.2-28.6] vs. 20.6 [18.3-23.2] Hz, p=0.007) compared to infants whose mothers did not smoke; with no difference between groups for R and I. Ethnicity and maternal HIV infection did not have a significant effect on any impedance measures.

Results of the multivariate analysis of respiratory impedance determinants are displayed in Table S1 in the online supporting information. Adjusted for body size, ethnicity, sex and maternal HIV status, infants whose mothers smoked during pregnancy had 0.35 mL.cm-H2O-1 lower C (95%CI -0.55 to -0.15, p=0.001) and 5 Hz higher fres (95%CI 1.9 to 8.1, p=0.002) compared to infants whose mothers did not smoke. Infants whose mothers were exposed to passive cigarette smoke during pregnancy also had reduced C, 0.23 mL.cmH2O-1 lower (95%CI -0.41 to -0.04, p=0.016) and 2.9 Hz higher fres (95%CI 0.09 to 5.7; p=0.04) compared to infants whose mothers were not exposed to passive smoke; although this effect was not as strong as the exposure to active maternal smoking. None of the exposures affected R or I measured with the FOT in the first few weeks of life.

**Discussion**

This is the first study to report the use of FOT in unsedated healthy term infants 1 to 3 months of age. The success rate of 93% is higher than has been described in other infant cohorts reporting lung mechanics measurements in unsedated infants.[17-20] The intra-test variability, ranging from 4% to 10% for resistance and from 7% to 22% for compliance, is similar to those reported in newborn infants [21] and older children [22] tested with the FOT. This suggests that FOT may be an appropriate lung function test for longitudinal studies through early childhood to adolescence. Further, this is the first investigation to report successful use of this technique in infants in a low–middle income setting, which carry a large burden of respiratory disease globally.[23]

Direct comparisons between resistance and compliance measured with FOT and that measured by other techniques in healthy unsedated infants such as the single occlusion technique (SOT),[24-26] cannot be made. In particular, the compliance obtained from the FOT measurements at frequencies much higher than the spontaneous breathing rate

is approximately 5 times lower than the quasi-static compliance measured by the SOT.[9 26] Since the respiratory system resistance is less frequency-dependent, the values of R determined in the present study with the FOT are similar to those obtained with the SOT. The R measured with FOT was slightly higher than that measured in unsedated European infants using the interrupter technique,[17] which may be partially explained by the differences in measurement technique used but may also reflect the different populations with differing risk of exposure in these studies. The FOT has the advantage of measuring Zrs during uninterrupted normal breathing pattern without the need of respiratory pauses, and hence possibly a more relevant measure of respiratory mechanics during tidal breathing.[27]

The strong inverse relationship observed between R and C (Fig. S3 in the online supporting information) reflects the size effects on the resistive and compliant mechanical properties, while the positive correlation between R and I indicates the large contribution of the upper airways to the respiratory impedance.

Male infants in the present study had a higher R and lower C as a group, compared to the female infants. This is consistent with previous studies that have shown male infants to have reduced early life lung function as compared to female infants.[26 28-32] Hanrahan et al. investigated healthy infants at 2 to 6 weeks with SOT and reported that male infants had a significantly higher R (0.083 vs. 0.079 $cmH_2O.s.mL^{-1}$; p=0.003) but a non-significantly lower C (5.4 vs. 5.54 $mL.cmH_2O^{-1}$; p=0.37) compared to female infants.[26] Stocks et al. tested preterm infants at 5 to 39 days with the SOT and multiple occlusion technique; female infants had a non-statistically significant lower resistance than males (95%CI: -2.93; p=0.2), and significantly higher time to peak expiratory flow over total expiratory time.[28] These sex differences in lung function early in life suggest that infant boys may have a less mature respiratory system at birth compared to girls. However, it has also been suggested in previous reports that postnatal growth and maturation may be faster in boys[26 28], which underlines the need for further investigations on the longitudinal changes of respiratory mechanics.

Infants of mothers who smoked during pregnancy in the present study had a significantly lower C compared to infants whose mothers did not smoke. The relationship between

maternal smoking and low infant lung function in early life is well established.[33] Forced expiratory flow was shown lower in infants whose mothers smoked during pregnancy,[32 34 35]; additionally respiratory compliance measured with SOT was reduced in infants at birth that had been exposed to in-utero tobacco smoke compared to infants without exposure (3.6 vs. 4.8 mL.cm$H_2O^{-1}$; $p<0.001$, 95% CI: 0.89 to 1.55).[36] The present cohort had a very high prevalence of maternal smoke exposure; 43% of infants studied had mothers who smoked during pregnancy, and a further 39% of babies had mothers who were exposed to environmental tobacco smoke. This is consistent with the low socio-economic status of the cohort, where most infants live in overcrowded conditions.

Thirty-three (19%) infants had HIV-infected mothers; all infants completed the prevention of mother-to-child transmission (PMTCT) program and no infants were found to be HIV infected. HIV-exposed but uninfected infants have a higher incidence of wheezing illness and respiratory infections in early life[37] and have an increased risk of pneumonia with treatment failure compared to unexposed uninfected infants.[38] Whether this is due to increased risk factor exposure, impaired immunity or effect of HIV exposure on early lung growth is not known. In this cohort, maternal HIV exposure had no effect on respiratory impedance at 6 weeks of age suggesting that low lung function at this early stage may not be the reason for increased pneumonia risk in early childhood; however, the data need to be interpreted with caution due to the small number of infants included and the exclusion of those with previous pneumonia. In addition, this cohort represents a group of HIV-infected woman in relatively good health, since they all received antiretroviral therapy as part of the prevention of mother to child transmission program.

## Conclusion

The present adaptation of the FOT is sensitive enough to detect the influences of sex and maternal tobacco exposure on respiratory mechanics in healthy unsedated infants. Further longitudinal studies of the impact of early-life environmental exposures on lung development and growth will be important in defining risk factors for respiratory disease.

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**Table 1. Demographics of infants (n=177)**

| | African ethnicity n=79 Median (25-75%) | Mixed African/other ethnicity n=98 Median (25-75%) | Total n=177 Median (25-75%) |
|---|---|---|---|
| Age (weeks) | 7.7 (7.0; 8.1) | 7.4 (6.7; 8.0) | 7.6 (6.9; 8.1) |
| Weight (kg) | 4.9 (4.6; 5.6) | 4.7 (4.3; 5.3) | 4.8 (4.4; 5.4)* |
| Weight for age z score | -0.2 (-0.7; 0.6) | -0.5 (-1.2; 0.3) | -0.36 (-0.98; 0.43)* |
| Length (cm) | 56 (53; 57.8) | 55 (53; 57) | 55 (53; 57) |
| Length for age z score | -0.7 (-1.8; 0.2) | -0.7 (-1.8; 0.1) | -0.7 (-1.8; 0.1) |
| Gestational age (week) | 39 (38; 40) | 39 (38; 40) | 39 (38; 40) |
| Birth weight (kg) | 3.1 (2.9; 3.5) | 3.0 (2.8; 3.5) | 3.1 (2.8; 3.5) |
| Birth weight z score | -0.5 (-1.3; 0.04) | -0.8 (-1.5; -0.1) | -0.7 (-1.4; -0.04) |
| Birth length (cm) | 50 (48; 53) | 50.5 (48; 53) | 50 (48; 53) |
| Birth length z score | 0.0 (-0.8; 1.0) | 0.1 (-1.1; 0.9) | -0.0 (-0.9; 0.9) |
| | **n (%)** | **n (%)** | **n (%)** |
| Male | 38 (48) | 51 (52) | 89 (50) |
| Maternal HIV (positive) | 26 (33) | 7 (7) | 33 (19)* |
| Maternal smoking | | | |
| Active smoker | 16 (20) | 59 (62) | 75(43)* |
| Passive smoke exposure | 39 (50) | 28 (30) | 67 (39)* |

* statistically significant difference, p<=0.05

**Table 2: Respiratory impedance parameters (n=164)**

| | Mean (SD) | Median (25-75%) | CoV [median (25-75%)] |
|---|---|---|---|
| Resistance (cmH2O.s.L-1) | 52.8 (19.1) | 50.2 (39.5; 60.6) | 5.9 (3.6; 9.5) |
| Compliance (mL.cmH2O1) | 0.85 (0.42) | 0.78 (0.61; 0.99) | 13.9 (6.9; 22.1) |
| Inertance (cmH2O.s2.L-1) | 0.073 (0.044) | 0.062 (0.050; 0.086) | 14.0 (7.7; 24.0) |
| Resonance frequency (Hz) | 23.7 (6.4) | 22.1 (19.5; 26.5) | 7.5 (4.1; 11.9) |

CoV: intra-subject coefficient of variation

**Figure 1:**

Schematic representation of the forced oscillation equipment. A loudspeaker delivers the computer-generated multi-component forcing function. Pressure is measured at each end of a wave-tube (P1and P2) for the estimation of respiratory impedance, and a pneumotachograph is used to monitor tidal airflow (V'). A bias flow reduces the influence of equipment dead-space on the infant's breathing pattern.

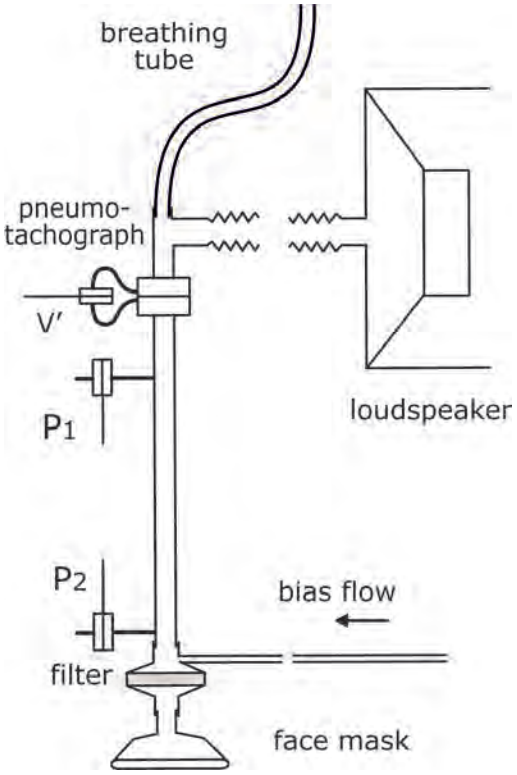
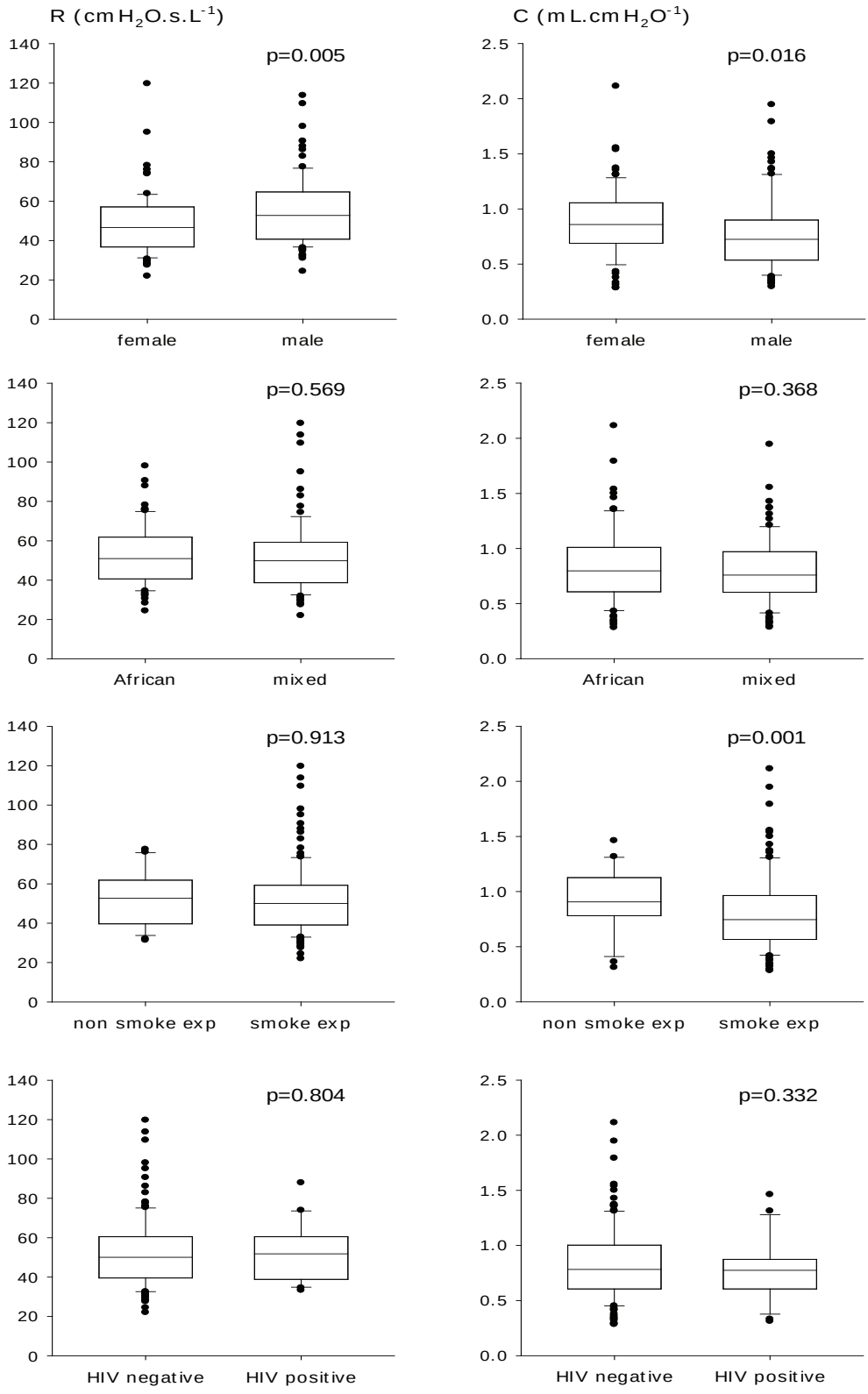


**Figure 2:**

Resistance (R) and compliance (C) by sex, ethnicity, maternal smoke exposure and maternal HIV. Data are shown as median, 25-75% and 95% confidence intervals.



## Supplementary information

### Study population

Healthy infants between 6 and 10 weeks of age enrolled in the Drakenstein Child Lung Health study (DCHS),[16] were eligible for the lung function measures. The DCHS study is a multidisciplinary birth cohort investigating the epidemiology and aetiology of childhood respiratory illness and the determinants of child respiratory health in a peri-urban area in South Africa. The primary aim is to investigate aetiology, progression and risk factors for childhood pneumonia and the impact on child health. The DCHS is located in the Drakenstein area in the town of Paarl, a peri-urban area, 60 km outside Cape Town, South Africa with a population of approximately 200,000. The local economy is based around commercial agriculture and light industry. More than 90% of the population access health care in the public sector including antenatal and child health services. The public health system is comprised of 23 primary health care clinics and one centralized hospital, Paarl Hospital, where all births and all hospital based paediatric care, including admissions occur. Similar to many low and middle income countries, the area has a high burden of childhood diseases and pneumonia [39] and a high prevalence of risk factors associated with pneumonia or severe disease, such as tobacco smoke exposure, malnutrition or poverty. Pregnant women were recruited from two primary health care clinics: Mbekweni, of predominantly African ethnicity and Newman predominantly of mixed African/other ethnicity. This is a peri-urban, accessible, low socioeconomic community. Mother-infant dyads were enrolled at 20-28 weeks' gestation and will be followed until children reach 5 years of age.

Lung function was tested at 6 weeks of age and is scheduled for 1 year of age and annually until 5 years. The first 219 infants presenting for FOT testing at the 6-week lung function visit were included in the current study. Of these, infants born premature (<37 weeks) and those who had previously had a lower respiratory tract infection were excluded from the current study. A further 13 infants were excluded for other reasons detailed in Fig. S1. The study was approved by the Faculty of Health Sciences, Human Research Ethics Committee, The University of Cape Town (401/2009) and by the Western Cape Provincial Health Research Committee. Mothers gave informed, written consent in their

first language for their infants to participate.

### Collection of antenatal and early life data

Maternal smoking was confirmed by a quantitative analysis of maternal urine cotinine (IMMULITE 2000 Nicotine Metabolite analyser, Siemens, Los Angeles, USA). Maternal urine specimens were collected antenatally and at birth. Smoking exposure based on maternal urine cotinine was defined as follows: active smoker, urine cotinine level of >500 $ng.mL^{-1}$; passive smoker, 11- 500 $ng.mL^{-1}$ and non-smoker <10 $ng.mL^{-1}$.[40] Following public sector protocol, all mothers would have had an HIV test at antenatal booking visit and repeated at delivery. Testing is done by rapid HIV immunoassay (Determine® HIV-1/2, Abbott, Illinois, USA) with a confirmatory ELISA test if the rapid test is positive (Enzygnost® Anti-HIV 1/2 Plus, Siemens Healthcare Diagnostic Products, Marberg, Germany). All HIV-exposed children had an HIV PCR test (Cobas Ampliprep system, Roche Molecular Systems, New Jersey, USA) done by 6 weeks of age.

### Model analysis of impedance

From the measurements of total respiratory impedance (Zrs) the parameters of respiratory mechanics were evaluated by fitting a resistance (R) – compliance (C) - inertance (I) model to the measured data (Fig. S2). Resonance frequency (fres), the zero crossing of the imaginary part of Zrs (Xrs), was calculated as $fres=1/(2\pi\sqrt{CI})$. R was obtained as the mean value of R between 12 and 32 Hz; the R values at 8 Hz were omitted from the estimation of R because of systematically higher values reflecting the contribution of tissue resistance in these young infants.1 The R values at >32 Hz were also excluded because of occasional elevations in R as a consequence of observed distortion of the parabolic airflow profile.41 In order to obtain a balanced contribution from the elastic and inertive properties of Xrs to the fitting, C and I were estimated from the Xrs data between 8 and 32 Hz whenever fres was ≤20 Hz and between 8 and 48 Hz otherwise.

### Multivariate analysis of determinants of impedance parameters

The association between the Zrs parameters and anthropometric indices, ethnicity, gender; exposure to active and passive maternal smoking and maternal HIV infection were tested in a multivariate linear regression model. Factors showing significant association

at a level of p=0.1 on initial univariate analysis (weight, gender and smoke exposure) and possible confounders (height, ethnicity, maternal HIV infection) were included in a multivariate linear regression model. The data are presented as coefficients, 95% CI and corresponding p-values in Table S1. Interactions were examined through stratification by gender. No evidence of interaction effects between covariates was found. However the study was underpowered for a comprehensive stratification analysis as subgroups were very small in sample size, but this was not the primary aim of the analysis.

**Table S1: Multivariate analysis of lung function, anthropometry, gender, ethnicity, maternal HIV infection and maternal smoking**

| **RESISTANCE ($cmH_2O.s.L^{-1}$)** | | | | |
|---|---|---|---|---|
| | Coefficient | 95% CI1 | | p-value |
| Weight-for-age z score2 | 4.23 | 0.48 | 7.99 | 0.027 |
| Length-for-age z score | -0.99 | -3.76 | 1.77 | 0.479 |
| Male | 8.62 | 2.60 | 14.64 | 0.005 |
| Ethnicity - African | -2.05 | -9.14 | 5.04 | 0.569 |
| HIV | 1.06 | -7.37 | 9.49 | 0.804 |
| Smoking - Passive | -0.47 | -9.02 | 8.07 | 0.913 |
| Smoking - Active | 0.35 | -8.96 | 9.67 | 0.941 |
| **COMPLIANCE ($mL\ cmH_2O^{-1}$)** | | | | |
| | Coefficient | 95% CI | | p-value |
| Weight-for-age z score | -0.018 | -0.099 | 0.063 | 0.667 |
| Length-for-age z score | 0.002 | -0.058 | 0.061 | 0.959 |
| Male | -0.161 | -0.291 | -0.030 | 0.016 |
| Ethnicity - African | -0.070 | -0.223 | 0.083 | 0.368 |
| HIV | -0.090 | -0.272 | 0.093 | 0.332 |
| Smoking – Passive | -0.227 | -0.412 | -0.042 | 0.016 |
| Smoking - Active | -0.352 | -0.553 | -0.150 | 0.001 |
| **INERTANCE ($cmH_2O.s^2.L^{-1}$)** | | | | |
| | Coefficient | 95% CI | | p-value |
| Weight-for-age z score | 0.007 | -0.002 | 0.015 | 0.144 |
| Length-for-age z score | 0.000 | -0.006 | 0.007 | 0.971 |
| Male | 0.006 | -0.008 | 0.021 | 0.367 |
| Ethnicity - African | -0.010 | -0.027 | 0.006 | 0.219 |
| HIV | 0.002 | -0.017 | 0.022 | 0.819 |
| Smoking - Passive | -0.010 | -0.030 | 0.010 | 0.306 |
| Smoking - Active | -0.010 | -0.032 | 0.012 | 0.375 |
| **RESONANCE FREQUENCY (Hz)** | | | | |
| | Coefficient | 95% CI | | p-value |
| Weight-for-age z score | -0.831 | -2.068 | 0.406 | 0.186 |
| Length-for-age z score | -0.145 | -1.056 | 0.766 | 0.753 |
| Male | 1.171 | -0.811 | 3.153 | 0.245 |
| Ethnicity - African | 1.414 | -0.922 | 3.750 | 0.234 |
| HIV | 0.042 | -2.734 | 2.818 | 0.976 |
| **Smoking - Passive** | **2.901** | **0.086** | **5.716** | **0.044** |
| **Smoking - Active** | **5.016** | **1.948** | **8.084** | **0.002** |

[1] 95% confidence intervals; [2] weight and height z-scores based on the WHO Child Health Standards 41

**Figure S1 Chart of the study population and exclusions.**

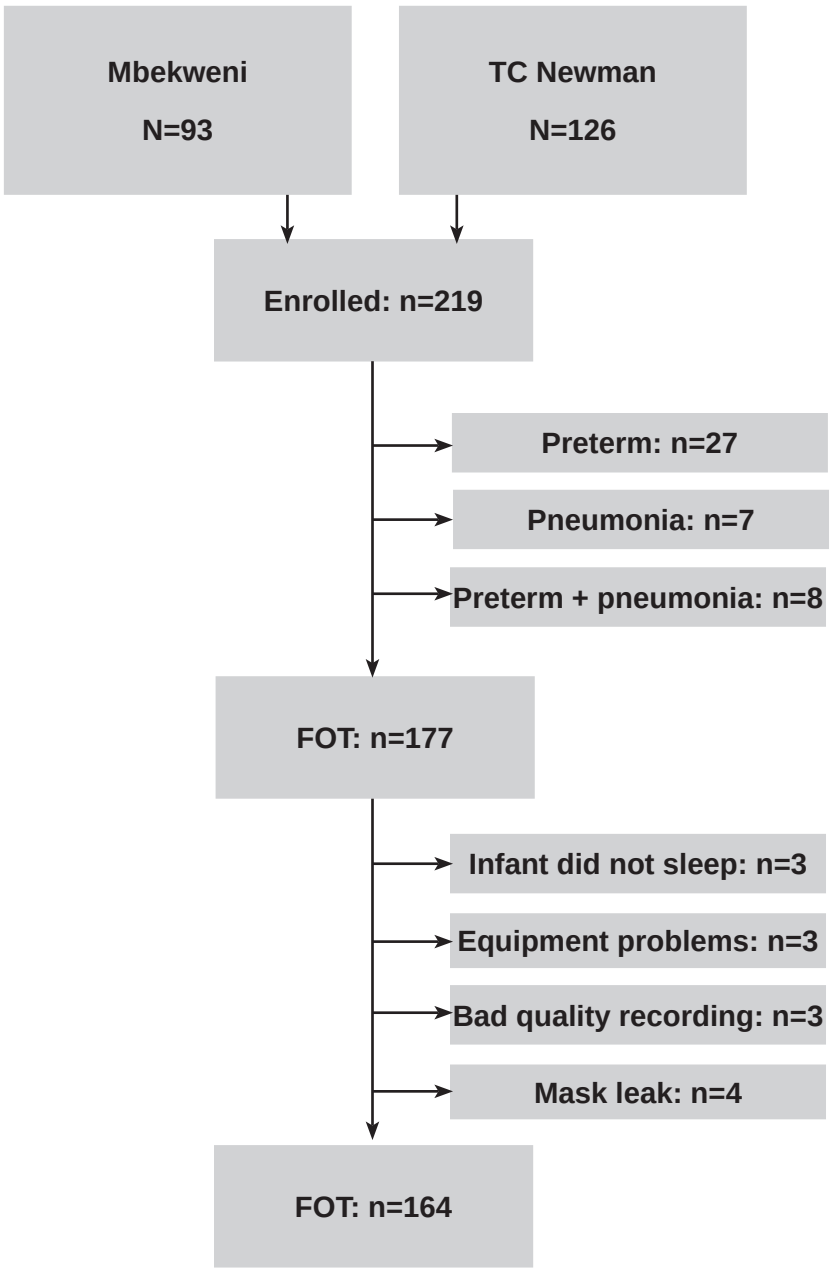


**Figure S2**

Schematics of the model fitting to the Zrs spectra represented by the real part or resistance (Rrs, top) and imaginary part or reactance (Xrs, bottom). Left: fitting the Xrs data in the 8-48-Hz range; right: curtailed frequency range for Xrs data with resonance frequency < 20 Hz.

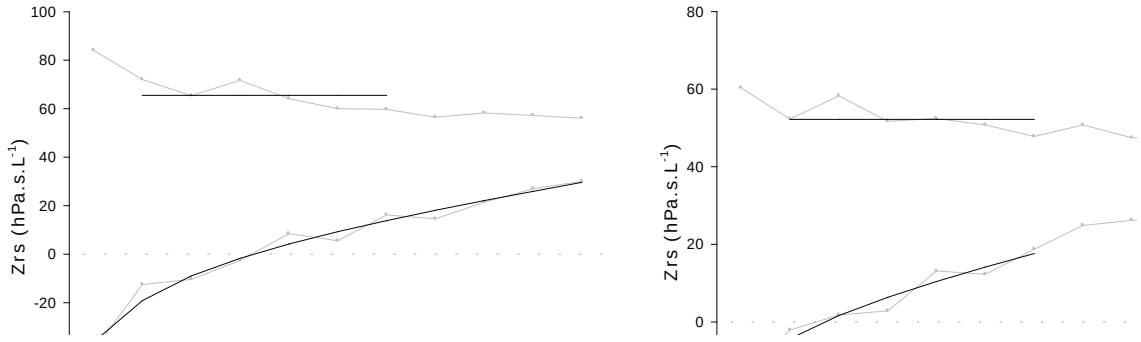
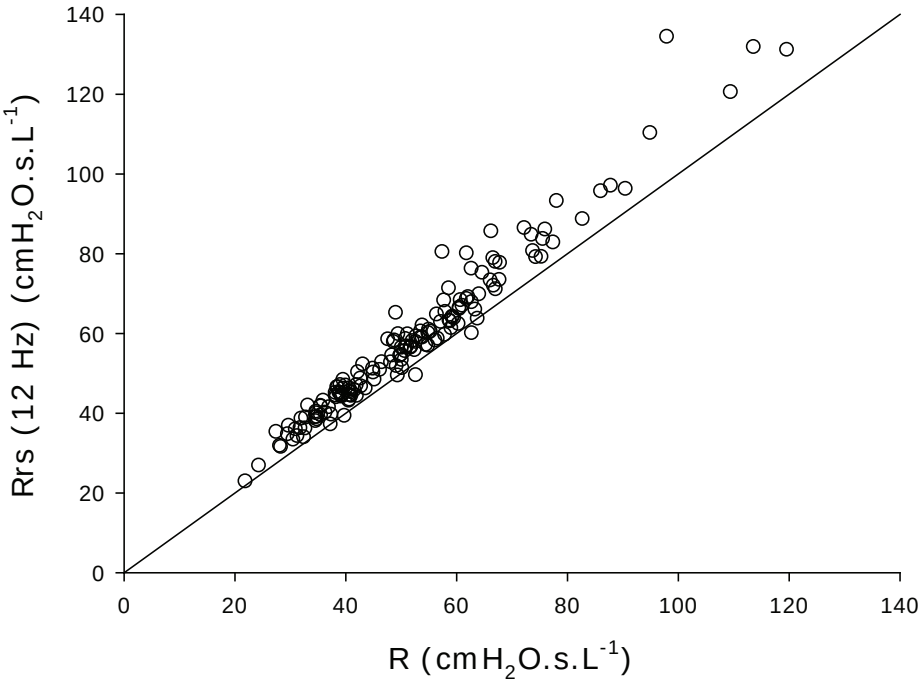


**Figure S3**

Respiratory system resistance (Rrs) at 12 Hz vs. mean resistance (R) from model fitting (top); effective compliance calculated from the reactance at 8 Hz vs. compliance (C) from model fitting (bottom). Lines of identity are shown.



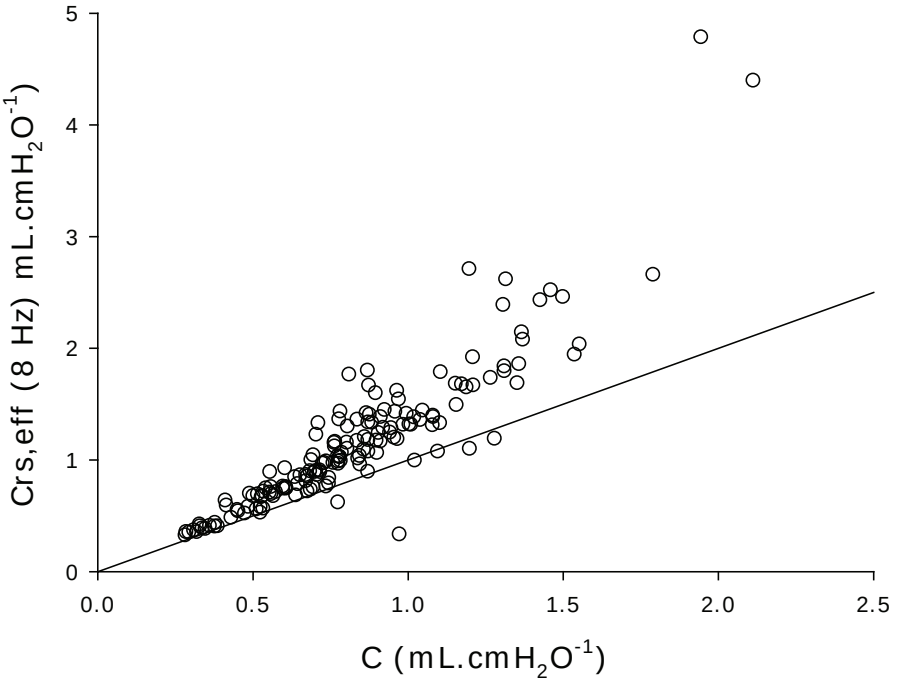
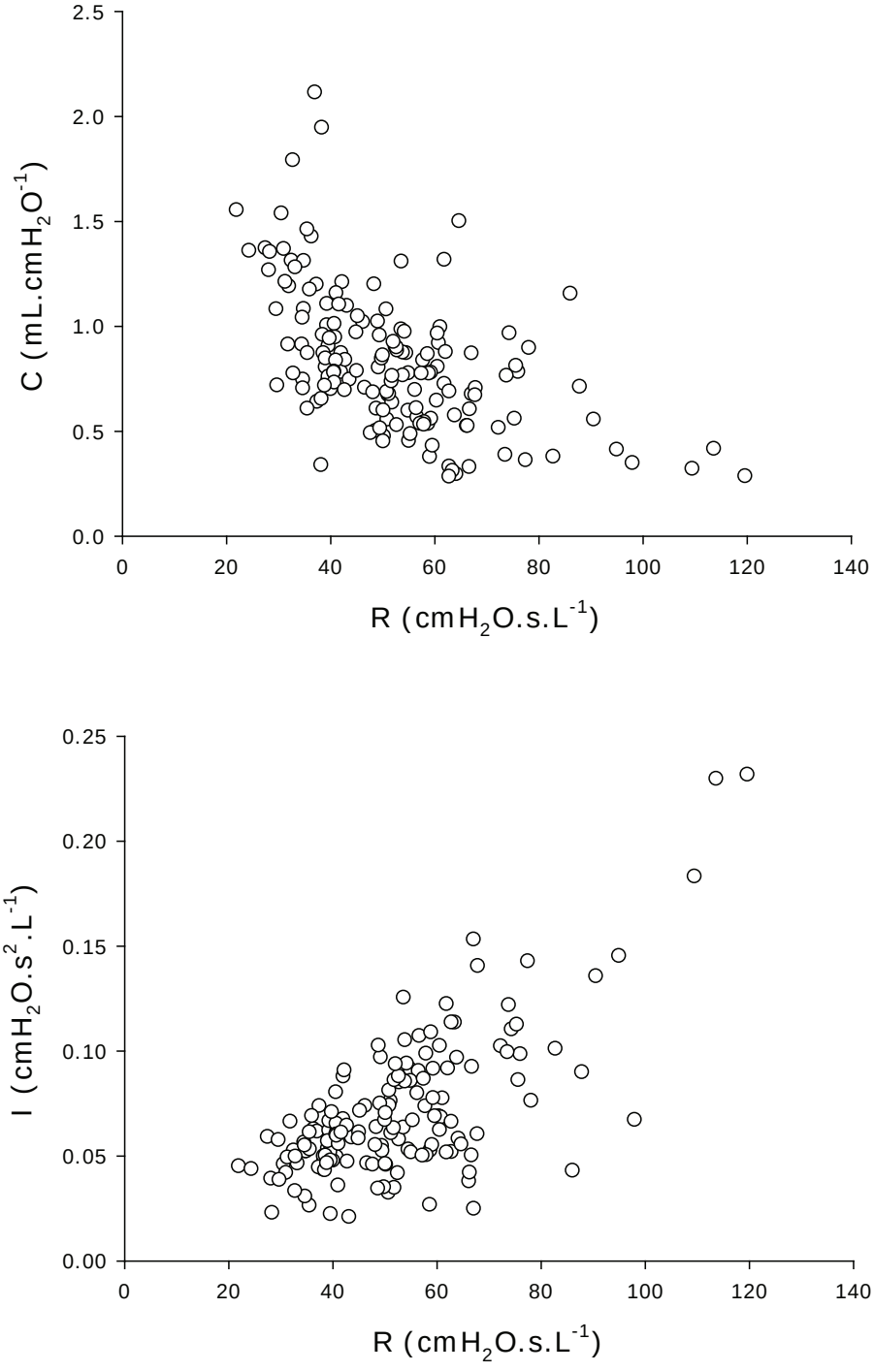



**Figure S4**

Relationships between the compliance (C, top) and inertance (I, bottom) and the resistance (R) of the total respiratory system. Symbols correspond to the mean values from each infant measurement.



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Chapter 4

# Lung function and exhaled nitric oxide in healthy unsedated African infants

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## Abstract

**Background:** Population appropriate lung function reference data are essential to accurately identify respiratory disease and measure response to interventions. There are currently no reference data in African infants.

**Aim:** To describe normal lung function in healthy African infants.

**Method:** Lung function was performed on healthy infants enrolled in the Drakenstein Child Health Study in South Africa. Infants were excluded if they were born preterm or had a history of neonatal respiratory distress or prior respiratory tract infection. Measurements, made during natural sleep, included the forced oscillation technique, tidal breathing, exhaled nitric oxide and multiple breath washout measures.

**Results:** Three hundred and sixty three infants were tested. Acceptable and repeatable measurements were obtained in 356 (98%) and 352 (97%) infants for tidal breathing analysis and exhaled nitric oxide outcomes, 345 (95%) infants for multiple breath washout and 293/333 (88%) infants for the forced oscillation technique. Age, sex and weight-for-age z score were significantly associated with lung function measures.

Conclusion: This study provides reference data for unsedated infant lung function in African infants and highlights the importance using population specific data.

**Short title:** Lung function in African infants

## Introduction

Measuring lung function in early life allows assessment of the determinants of early respiratory health and may provide a prognostic and susceptibility measure of respiratory disease. This is especially relevant to low and middle income settings (LMICs), where there is a very high burden of childhood respiratory disease, however there is very limited data on infant lung function in these areas. Moreover it is now well established that early lung function tracks through to adult hood, predicting diminished lung function and chronic respiratory disease in later life. [1 2]

Appropriate lung function data are essential to accurately assess the impact of early life factors on lung function and to distinguish between health and disease. Using reference data developed from differing equipment or methods or derived from different populations can lead to misinterpretation of the test results [3]. Hence developing reference ranges for tests and for a specific population is important. Non-invasive lung function measures undertaken in unsedated infants have been developed [4-8] furthering the use of infant lung function testing in an epidemiological setting and the development of robust reference ranges. Reference data for Caucasian infants for both sedated [9-13] and unsedated [14 15] lung function tests have been published. Reference data for infant lung function outcomes in non-Caucasian subjects [16] are limited and no data are available for African infants, nor for infants from LMICs.

This study aimed to describe the lung function in healthy South African infants living in low socio-economic conditions and to provide reference data for tidal breathing parameters, exhaled nitric oxide, multiple breath washout and forced oscillation technique measures.

## Methods

### Setting

Infant lung function testing was undertaken as part of a birth cohort study, the Drakenstein Child Health study (DCHS).[17] This birth cohort study in a peri-urban, low socio-economic community in South Africa, aims to investigate the epidemiology and aetiology of childhood respiratory illness and the determinants of child lung health. The lung function testing site was established at the local hospital.

### Participants

Healthy infants, enrolled in the DCHS, underwent testing at 5 to 11 weeks. Infants were excluded if they were born preterm (<37 weeks gestation), had a history of respiratory distress at birth or prior respiratory tract infection. Gestation was assessed by antenatal ultrasound or by a combination of last menstrual period, antenatal examination of pubic symphysis fundal height and birth weight, when ultrasound was not available.

### Antenatal and early life data

Information regarding antenatal, birth and early life exposures and events were collected by questionnaire at scheduled antenatal and study visits.

### Lung function measurements

Lung function measurements included tidal breathing (TBFVL), exhaled nitric oxide (eNO), sulphur hexafluoride multiple breath washout (MBW) and the forced oscillation technique (FOT). Infants were tested from July 2012 to Dec 2013 for TBFVL, eNO and MBW and, for operational reasons, from October 2012 to Dec 2013 for FOT. Lung function measures were taken in unsedated infants during behaviourally assessed quiet sleep, as previously published [18 19]. A size 0 round infant silicone mask (Laerdal silicone mask, Laerdal Medical, Stavanger, Norway) for the TBFVL, eNO and MBW tests and a size 0 rigid anaesthetic mask (Neonate crystal anaesthesia mask, no. 39170, Koo Asia, Hong Kong) was used for the FOT. All testing conformed to ATS/ERS guidelines. [20-22]

**Tidal breathing and exhaled nitric oxide measures** were collected simultaneously

using the Exhalyser D with ultrasonic flow meter and CLD 88 Exhalyzer chemoluminescent analyser (Ecomedics AG, Duernton, Switzerland) as described previously. [6 18] A 90 second measurement epoch was made during quiet tidal breathing a minimum of 30 seconds after initial mask placement. Recordings were included if >30 consecutive breaths (free of sighs, respiratory pauses, irregular volume breaths or air leak) of tidal breathing were recorded and analysed according to international guidelines.[23] Mean tidal breathing measures, eNO and NO output were calculated using analysis software (Wbreath v3.28.0. Ndd Medizintechnik, AG, Zurich, Switzerland).

**Multiple breath washouts (MBW)** were performed using 4% SF6 as a tracer gas and ultrasonic flow meter (Spirison®, Ecomedics, Duernten, Switzerland) with acquisition and analysis software (Wbreath v3.28.0, Ndd Medizintechnik AG, Zurich, Switzerland) as reported previously. [5 18 24] The washout period began after a ten breath equilibrium period was obtained at the end of the tracer gas wash in. The washout continued until the tracer gas was eliminated from the lungs. The recordings were defined as acceptable for analysis if they were free of leak, occurred during quiet sleep; the wash-in equilibrium period had a stable tidal volume and a variation of inspiratory and expiratory end tidal inert gas concentration of <1%; there were no sighs, breath holds or irregular breathing pattern within 10 breaths of the wash-in plateau or 10 breaths after the SF6 concentration had returned to baseline, 1/40$^{th}$ the concentration at start of washout.[22] Three successful recordings were taken. Test repeatability was defined as functional residual capacity (FRC) means within 25% and lung clearance index (LCI) within 1 turnover of each other. The mean FRC, LCI and moment ratios of the three tests were reported. If only two successful tests were obtained the tests were reported as the mean of two tests if the FRC mean was within 10% of the lower value and the LCI were within 1 turnover of each other. MBW indices such as LCI are related to an individual's underlying respiratory patterns, including the respiratory rate, tidal volume and FRC. [25 26] Moment ratios describe the skewness of the washout curve and may be less sensitive to the effect of these underlying factors.[27] Mean dilution numbers, M0, M1 and M2 are calculated from the area under the curve of the end tidal inert gas concentration and lung turnovers measured.[22] The first moment ratio was calculated as M1/M0 and the second as M2/M0, using the automated mode within the Wbreath software.

The **forced oscillation technique (FOT)** measurement was made with purpose built equipment. (University of Szeged, Hungary), as previously reported.[19 28] A composite medium frequency signal (8-48Hz, peak-to-peak pressure of 2 cm $H_2O$) was delivered to the infants via a 10 or 20cm wave-tube through a facemask covering the mouth and nose. A minimum of 5 technically acceptable 30-s data epochs was collected. Recordings (or short segments of them) that contained breath holds, cries, irregular breathing or leaks around the facemask were excluded. The epochs required at least 10 regular consecutive breaths, free from sighs or breath holds, to be included in analysis. Zrs spectra were defined as reproducible if at least 3 of the spectra had Zrs magnitude values within 10% of each other. The mean values of respiratory system impedance (Zrs) spectra were evaluated by fitting a resistance (R) – compliance (C) - inertance (I) model to the measured data.[28] Resonance frequency ($f$res) was calculated as $f\text{res}=1/(2\pi\sqrt{CI})$.

### Ethics

The study was approved by the Faculty of Health Sciences, Human Research Ethics Committee, University of Cape Town (401/2009) and by the Western Cape Provincial Health Research Committee. Mothers gave informed, written consent in their first language for their infants to participate.

### Statistical analysis

Descriptive statistics were performed using STATA 13 for windows (STATA Corporation, College Station, Texas, USA). Data are presented as mean and standard deviation (SD) for normally distributed variables and median and 25-75% confidence intervals for non-normally distributed variables. Comparisons between populations are presented as sample means and standard error. The intra-subject coefficient of variation (CoV) was calculated as the ratio of each parameter standard deviation over the parameters mean per study participant i.e. for the TBFVL and eNO this is the mean value of included breaths and for MBW and FOT the mean of the results from each test. The median and IQR of the CoV are reported.

Reference equations were fitted using a stepwise linear regression model with the significance level for removal set to significance levels above 0.05. The linear predictions

from the reference equations were used to determine the predicted values for each lung function measurement. Details of statistical analysis are given in the online supplement.

## Results

Of the 448 infants, 85 infants were excluded (29 had had a prior lower respiratory tract infection and 56 were preterm), giving 363 infants eligible for inclusion, **figure 1**. The median age of infants was 7.4 weeks with an equal gender distribution, **table 1**. Demographic and socio-economic factors are shown in **table 2**. The demographics by enrolment site are shown in **table E1**. Success rates for testing were 356 (98%) of tidal breathing tests, 352 (97%) eNO and 345 (95%) of MBW tests; and for the FOT, 293/333 (88%). Reasons for unsuccessful testing are detailed in **figure 1**.

### Tidal breathing

The tidal breathing group outcomes are listed in **table 3**. The CoV ranged from 7.1% for minute ventilation to 20% for the ratio of time to peak tidal expiratory flow over total expiratory time ($t_{PTEF}/t_E$). The association with known and possible predictors of tidal breathing parameters are shown in **tables E2-7** in the online supplement. Weight-for-age z score was positively associated with tidal volume, minute ventilation and mean tidal flows; and negatively associated with respiratory rate. Weight was not a predictor of $t_{PTEF}/t_E$. Males had a slightly increased tidal volume (2.3 mL higher compared to females; 95% CI 1.2 to 3.3, p<0.001) and lower $t_{PTEF}/t_E$ (2.8% lower compared to females; 95% CI -5.3 to -0.3, p=0.03).

### Exhaled nitric oxide

Group results for measured eNO and NO output are detailed in **table 3.** The median intra-subject CoV was small; 4.3% for eNO and 8.0 % for NO output. Associations are shown in **tables E8 and 9**. Age had a small but significant positive association with both eNO and NO output. Weight had a weak association with eNO but not with NO output. Sex was not associated with NO measures.

### Multiple breath washout

The group results for FRC, the 1$^{st}$ and 2$^{nd}$ moment ratios (M0/M1 and M0/M2) and LCI

are detailed in **table 3**. The median intra-subject CoV was small for MBW measurements, FRC 5.4%, LCI 4.0%, M0/M1 3.8% and M0/M2 8.4%. The results of the multivariate analysis for associations of MBW measurements are shown in **tables E10-13**. Age, weight-for-age z score and birth weight z score were associated with FRC in the multivariate analysis. No size associations were found for the moment ratios or LCI. No predictive equation was appropriate for the moment ratios or LCI and the predicted upper and lower limits of normal were defined using the observed mean and standard deviation as opposed to the predicted.

### Forced oscillation technique

The FOT group outcomes are detailed in **table 3**. The median intra-subject CoV was 4.9% for resistance, 11% for compliance and 5.4% for *f*res. Associations are shown in **tables E14-16**. Length was positively associated with resistance, compliance and *f*res. Weight was positively associated with resistance. Male sex was associated with increased resistance, decreased compliance and a slightly higher *f*res. Stratification by sex was considered but did not lead to meaningful differences in reference equations.

The South African reference equations calculated from these data are listed in **table E17**. The observed data were compared with the values predicted by these reference equations and the published European reference equations [15] in **table 4**. The European prediction equations did not satisfactorily predict the South African values.

### Discussion

This large cohort study provides reference data for lung function measures in healthy unsedated South African infants early in life, providing reference equations that can be used in clinical and epidemiological studies in African children. In addition this study provides the first reference data for a novel forced oscillation technique in healthy 6-week-old infants, a safe and feasible measure of respiratory system impedance in unsedated infants.

Strengths of this study include the large size of the cohort, the collection of data using rigorous methodology by the same trained staff under similar conditions, which adhered to ATS/ERS guidelines; the strict quality control during data collection and analysis and

the high success rate of testing. Factors contributing towards a high success rate were dedicated quiet testing space with a well trained and experienced team skilled in co-ordinating infant sleep and testing; participant families being willing to wait at the testing site for infant sleep and the fact that repeat testing was attempted in failed cases.

A limitation of the study is the narrow age range in which lung function was measured, which limits generalizability of these reference data to older children. However this reference data may be useful in epidemiological studies and in longitudinally assessing the impact of early life exposures on lung health. Children from LMICs are at particular risk of respiratory disease,[29] hence understanding the impact of early exposures on lung growth and function in these settings is key.

### Comparison with data from high-income settings

There are few data on lung function parameters in healthy unsedated infants using the same techniques. Similar tidal breathing, exhaled nitric oxide and multiple breath washout data have been published in European and Australian infants. [15 30]

### Tidal breathing

The intra-test intra-individual coefficients of variation (CoV), a measure of test repeatability, of the TBFVL measures were similar to that previously reported using the same techniques in 6-week-old infants.[8 15] Tidal volume, respiratory rate and flows were similar to those previously reported in sedated and unsedated infants, although South African infants had a slightly larger tidal volume and minute ventilation compared to European infants.[15 30] However, South African infants were an average of 2 weeks older and 0.5kg heavier than the European infants, accounting for this difference.[15 30]

### Exhaled nitric oxide

The exhaled nitric oxide was lower in South African compared to European infants, mean ± SD eNO of 10.1 ± 6.8 in South African vs. 14.3 ± 6.0 European infants.[15]

### Multiple breath washout

South African infants had a lower FRC and higher LCI (poorer gas mixing efficiency)

compared to European and American studies. [15 31] Fuchs et al reported measures using the same techniques in European infants of a similar age (mean ± SD 7.2 ± 0.4 weeks vs. 6.75 ± 0.6 weeks in European infants). South African infants had a significantly lower FRC compared to European infants (mean ± SD FRC 78 ± 17 mL vs. 102 ±16 mL in European infants). These differences in MBW results may be accounted for in part by differing hardware and software, but may also represent different populations with particular exposures. These data suggest that infants living in low socio-economic conditions with high exposure to air pollutants such as tobacco smoke, have early evidence of impaired lung growth and function compared to infants from high income areas.

**Forced oscillation technique**

No studies have been published of normative data in 6-week-old infants using this version of the FOT. However the resistance (R) and compliance (C) CoV are similar to those collected using the FOT in newborn infants [28] and older children.[32] Direct comparisons cannot be made between R and C measured with FOT and other techniques, such as the interrupter technique (IT) and single breath occlusion technique (SOT). However R, which is relatively frequency independent, is similar to that measured with SOT.[11 33] In contrast C measured with FOT is lower than that measured with the SOT.[11 33] R measured here was slightly higher than R measured in unsedated European infants using the IT.[15] Differing measurement techniques may have contributed to the reduced impedance in South African infants, but may also be due to population differences.

**Effect of sex on lung function variables**

The reduced flow ratio found in males is consistent with previous reports of lower lung function in male as compared to female infants.[34-37] Male infants had a lower compliance, higher resistance and higher resonant frequency. This is similar to previous reports of lower compliance in healthy male compared to female infants.[11]

The previously published equations in Caucasian European infants did not fit the South African data well, despite the data being collected under similar conditions. This highlights the importance of reference data being specific not only for the equipment and method used, but also for the population studied.

## Conclusions

This paper is the first description of healthy reference ranges of lung function data in African infants. These data provide reference equations for lung function measures in unsedated 6-10 week African infants. These data highlight the importance of using reference data that is specific to the population studied.


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**Figure 1: Cohort description before exclusions (N= 448)**

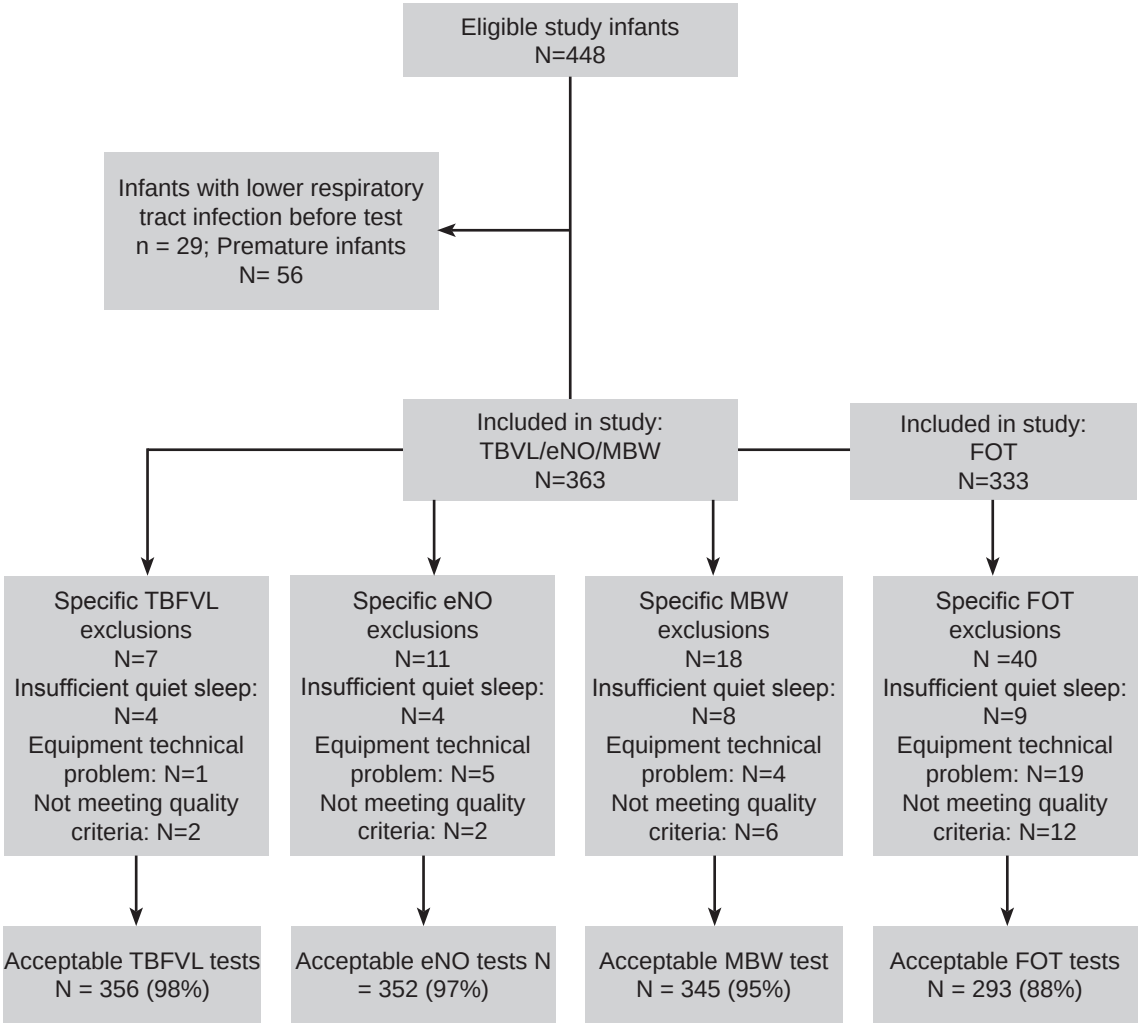


**Table 1: Anthropometric characteristics (N = 363)**

| | **Median** | **25-75%** | **Range** |
|---|---|---|---|
| Age (weeks) | 7.4 | 6.6 – 8.1 | 5.3 to 11.4 |
| Weight (kg) | 4.8 | 4.4 – 5.4 | 2.8 to 6.9 |
| Weight for age z score[1] | -0.32 | -0.97 – 0.44 | -4.2 to 2.1 |
| Length (cm) | 55 | 53 - 57 | 47 to 63 |
| Height for age z score | -0.66 | -1.7 – 0.2 | -5.2 to 2.8 |
| Gestational age (weeks) | 39 | 38 - 40 | 37 to 43 |
| Birth weight (kg) | 3.1 | 2.8 – 3.5 | 1.8 to 4.3 |
| Birth weight z score | -0.7 | -1.4 to -0.1 | -3.7 to 1.6 |
| Birth length (cm) | 51 | 48 - 53 | 35 to 58 |
| Birth length z score | 0.003 | -0.8 - 0.9 | -7.1 to 3.3 |

[1] Z scores calculated using the WHO Child Growth Standards[38] and updated Fenton new-born growth charts[39]

**Table 2: Demographics and socio-economic characteristics of participants**

| | **Total (N=363)**<br>**N (%)** |
|---|---|
| Male Sex | 181 (50) |
| Maternal HIV infection | 69 (19) |
| Maternal smoking in pregnancy | 121 (34 |
| Caesarean section | 69 (19) |
| Exclusively breastfed | 148 (41) |
| Maternal SES | |
| Lowest SES | 97 (27) |
| Low-Moderate SES | 85 (23) |
| Moderate-high SES | 94 (26) |
| High SES | 87 (24) |
| Ethnicity | |
| African | 176 (48) |
| Mixed ethnicity | 187 (51) |

**Table 3: Lung function values in healthy South African infants**

| | Mean (SD) | Range | Median<br><br>(25-75%) | CoV<br><br>med (25-75%) |
|---|---|---|---|---|
| **Tidal breathing parameters n=356** | | | | |
| Tidal Volume mL | 34.9 (6.3) | 18.86 – 54.24 | 34.52 (30.78 – 39.0) | 7.7 (6.3 – 9.9) |
| Respiratory Rate $n.min^{-1}$ | 48.1 (11.9) | 24.8 – 104.10 | 46.4 (40.10 – 54.25) | 8.0 (6.7 – 10.2) |
| Minute ventilation $mL.min^{-1}$ | 1627.0 (307.6) | 986.5 – 2836.0 | 1598 (1398 – 1810) | 7.1 (5.9 – 9.0) |
| Mean inspiratory tidal flow $mL.s^{-1}$ | 60.6 (10.6) | 34.85 – 102.0 | 59.2 (53.4 – 66.5) | 7.7 (6.2 – 10.3) |
| Mean expiratory tidal flow $mL.s^{-1}$ | 50.0 (11.96) | 25.00 – 92.93 | 48.3 (41.0 – 56.9) | 9.1 (7.4 – 11.5) |
| $t_{PTEF}/t_E$ % | 39.8 (12.1) | 12.87 – 82.17 | 39.9 (31.6 – 46.7) | 20.1 (16.8 – 25.2) |
| **Exhaled nitric oxide n=352** | | | | |
| eNO ppb | 10.1 (6.8) | 0.20 – 46.20 | 9.0 (5.0 - 13.7) | 4.3 ( 3.4 – 6.5) |
| NO output $nL.min^{-1}$ | 33.1 (21.3) | 0.3 – 100.0 | 30.6 (16.5 – 47.5) | 8.0 (6.6 – 10.2) |
| **Multiple breath washout n=345** | | | | |
| FRC mL | 77.97 (17) | 47.3 – 162.7 | 75.1 (66.1 – 86.6) | 5.4 (3.3 – 7.8) |
| M0/M1 | 2.1 (0.1) | 1.5 – 2.6 | 2.1 (2.0 – 2.2) | 3.8 (2.2 – 5.5) |
| M0/M2 | 8.2 (1.1) | 3.9 – 11.9 | 8.1 (7.4 – 8.9) | 8.4 (4.9 – 12.4) |
| LCI | 7.2 (0.4) | 5.35 – 8.58 | 7.16 (6.91 – 7.46) | 4.0 (2.4 – 5.8) |
| **Forced oscillation technique n=293** | | | | |
| R $cmH_2O.s.L^{-1}$ | 48.6 (15.7) | 22.0 – 119.7 | 45.6 (38.1 – 57.2) | 4.9 (3.0 – 8.0) |
| C $mL.cmH_2O^{-1}$ | 0.95 (0.44) | 0.28 – 3.32 | 0.87 (0.68 – 1.15) | 11.0 (6.1 – 17.9) |
| *f*res Hz | 22.6 (6.1) | 10.7 – 42.0 | 21.2 (18.7 – 25.4) | 5.4 (3.3 – 9.6) |

CoV: intra-individual intra-test coefficient of variation, FRC: functional residual capacity, M0/M1: first mean dilution number, M0/M2: second mean dilution number, LCI: lung clearance index, $t_{PTEF}/t_{E:}$ ratio of time to peak tidal expiratory flow over total expiratory time, eNO: exhaled nitric oxide, R: respiratory system resistance, C: respiratory system compliance, *f*res: resonant frequency

**Table 4: Comparison between measured and predicted values for South African and European models**

| | Observed Values Mean (std error) | Predicted Values Mean (std error) | Reference Values[15] Mean (std error) |
|---|---|---|---|
| Tidal Volume mL | 34.90 (0.34) | 34.90 (0.21) | 33.39 (0.19) * |
| Respiratory Rate $n.min^{-1}$ | 48.11 (0.63) | 48.08 (0.20) | 45.93 (0.31) * |
| Minute ventilation $mL.min^{-1}$ | 1,627.08 (16.33) | 1,624.67 (5.86) | # |
| Mean inspiratory tidal flow $mL.s^{-1}$ | 60.55 (0.56) | 60.50 (0.23) | 57.44 (0.36) * |
| Mean expiratory tidal flow $mL.s^{-1}$ | 50.05 (0.63) | 49.96 (0.17) | # |
| $t_{PTEF}/t_E$ % | 39.73 (0.63) | 39.71 (0.15) | # |
| eNO ppb | 10.11 (0.36) | 10.14 (0.09) | 13.13 (0.15) * |
| NO output nL/sec | 8.94 (0.24) | 8.92 (0.05) | 3.70 (0.03) * |
| FRC mL | 78.01 (0.92) | 77.95 (0.34) | 107.18 (0.47)* |
| M0/M1 | 1.54 (0.01) | 1.54 (0.00) | # |
| M0/M2 | 5.92 (0.04) | 5.92 (0.01) | # |
| R $cmH_2O.s.L^{-1}$ | 48.55 (0.92) | 48.78 (0.19) | § |
| C $mL.cmH_2O^{-1}$ | 0.95 (0.03) | 0.94 (0.01) | § |
| *F*res Hz | 22.64 (0.36) | 22.65 (0.09) | § |

*statistically significant difference with $p < 0.001$; # no reference equations fitted; § not tested

## Supplementary information

### Statistical analysis

Statistical analyses were performed using STATA 13 for windows (STATA Corporation, College Station, Texas, USA). Weight and length for age z scores were calculated using the WHO Child Growth Standards “I grow up” STATA package.[38] Birth weight and birth length z scores were calculated using the Fenton 2013 growth charts for boys and girls based on gestational age, birth weight and length.[39] Reference equations were fitted using a stepwise linear regression model with the significance level for removal set to significance levels above 0.05. The linear predictions from the reference equations were used to determine the predicted values for each lung function measurement. Due to having a very skewed distribution, exhaled nitric oxide was also analyzed as a log transformed variable. However, the significant predictors resulting from the multivariate analysis (**table E7a**) of the transformed variable was not different to the analysis of the non-transformed variable (**table E7**), and hence the non-transformed variable analysis was used to develop a prediction equation, as the effect sizes are easier to interpret. These were compared to the European reference values [15] by fitting the published reference equations to the observed data. Significance testing between predicted values was done using two sample Student’s t-test with a 0.05 level of significance.

**Table E1: Demographics and socio-economic characteristics of participants by study site.**

| | Mbekweni (N=176) N (%) | Newman (N=187) N (%) | Total (N=363) N (%) |
|---|---|---|---|
| Male Sex | 81 (46) | 100 (54) | 181 (50) |
| Maternal HIV infection | 61 (35) | 8 (4) | 69 (19) |
| Maternal smoking in pregnancy | 26 (15) | 95 (52) | 121 (34 |
| Caesarean section | 37 (21) | 32 (17) | 69 (19) |
| Exclusively breastfed | 69 (39) | 79 (42) | 148 (41) |
| Maternal SES | | | |
| Lowest SES | 61 (35) | 36 (19) | 97 (27) |
| Low-Moderate SES | 48 (27) | 37 (20) | 85 (23) |
| Moderate-high SES | 41 (23) | 53 (28) | 94 (26) |
| High SES | 26 (15) | 61 (33) | 87 (24) |
| Ethnicity | | | |
| African | 174 (99) | 2 (1) | 176 (48) |
| Mixed ethnicity | 2 (1) | 185 (99) | 187 (51) |

**Table E2: Univariate and multivariate analysis for tidal volume**

| | | Univariate Model | | | | Multivariate model | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Coefficient | 95% CI | | p-value | Coefficient | 95% CI | | p-value | Adj $R^2$ | RSD* |
| Tidal Volume (mL) | | | | | | | | | | 37% | 5.07 |
| | Age at study date (weeks) | 1.830 | 1.211 | 2.449 | 0.000 | 1.619 | 1.095 | 2.142 | 0.000 | | |
| | Male sex | 1.678 | 0.368 | 2.988 | 0.012 | 2.166 | 1.092 | 3.329 | 0.000 | | |
| | Weight-for-age z score | 3.203 | 2.651 | 3.754 | 0.000 | 3.185 | 2.665 | 3.705 | 0.000 | | |
| | Length-for-age z score | 1.582 | 1.137 | 2.027 | 0.000 | | | | | | |
| | Birth weight z score | 2.160 | 1.528 | 2.791 | 0.000 | | | | | | |

* RSD: residual standard deviation

**Table E3: Univariate and multivariate analysis for respiratory rate**

| | | Univariate Model | | | | Multivariate model | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Coefficient | 95% CI | | p-value | Coefficient | 95% CI | | p-value | Adj $R^2$ | RSD* |
| Respiratory Rate ($n.min^{-1}$) | | | | | | | | | | 7% | 11.511 |
| | Age at study date (weeks) | -2.275 | -3.467 | -1.083 | 0.000 | -2.103 | -3.291 | -0.915 | 0.001 | | |
| | Male sex | 0.000 | -2.481 | 2.480 | 1.000 | | | | | | |
| | Weight-for-age z score | -2.392 | -3.580 | -1.204 | 0.000 | -2.294 | -3.471 | -1.119 | 0.000 | | |
| | Length-for-age z score | -1.584 | -2.459 | -0.710 | 0.000 | | | | | | |
| | Birth weight z score | -2.559 | -3.787 | -1.330 | 0.000 | | | | | | |

RSD: residual standard deviation

**Table E4: Univariate and multivariate analysis for minute ventilation**

| | | Univariate Model | | | | Multivariate model | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **Coefficient** | **95% CI** | | **p-value** | **Coefficient** | **95% CI** | | **p-value** | **Adj $R^2$** | **RSD*** |
| Minute Ventilation ($mL.min^{-1}$) | | | | | | | | | | 9% | 294.25 |
| | Age at study date (weeks) | 9.642 | -21.864 | 41.148 | 0.548 | | | | | | |
| | Male sex | 81.801 | 18.064 | 145.538 | 0.012 | 80.993 | 18.410 | 143.576 | 0.011 | | |
| | Weight-for-age z score | 77.319 | 47.072 | 107.566 | 0.000 | 102.115 | 65.752 | 138.479 | 0.000 | | |
| | length-for-age z score | 25.054 | 2.214 | 47.895 | 0.032 | | | | | | |
| | Birth weight z score | 15.116 | -17.449 | 47.682 | 0.362 | -41.165 | -79.072 | -3.258 | 0.033 | | |

* RSD: residual standard deviation

**Table E5: Univariate and multivariate analysis for mean tidal inspiratory flow**

| | | Univariate Model | | | | Multivariate model | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **Coefficient** | **95% CI** | | **p-value** | **Coefficient** | **95% CI** | | **p-value** | **Adj $R^2$** | **RSD*** |
| Mean tidal insp. flow ($mL.s^{-1}$) | | | | | | | | | | 15% | 9.780 |
| | Age at study date (weeks) | 0.585 | -0.497 | 1.667 | 0.288 | | | | | | |
| | Male sex | 3.904 | 1.732 | 6.076 | 0.000 | 4.334 | 2.264 | 6.404 | 0.000 | | |
| | Weight-for-age z score | 3.355 | 2.342 | 4.368 | 0.000 | 3.522 | 2.524 | 4.522 | 0.000 | | |
| | length-for-age z score | 1.385 | 0.609 | 2.160 | 0.001 | | | | | | |
| | Birth weight z score | 1.038 | -0.076 | 2.151 | 0.068 | | | | | | |

* RSD: residual standard deviation

**Table E6: Univariate and multivariate analysis for mean tidal expiratory flow**

| | | Univariate Model | | | | Multivariate model | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **Coefficient** | **95% CI** | | **p-value** | **Coefficient** | **95% CI** | | **p-value** | **Adj $R^2$** | **RSD** |
| Mean tidal exp. flow ($mL.s^{-1}$) | | | | | | | | | | 4% | 11.775 |
| | Age at study date (weeks) | 0.103 | -1.123 | 1.329 | 0.869 | | | | | | |
| | Male sex | 2.115 | -0.375 | 4.606 | 0.096 | | | | | | |
| | Weight-for-age z score | 2.103 | 0.903 | 3.303 | 0.001 | 2.979 | 1.525 | 4.435 | 0.000 | | |
| | length-for-age z score | 0.485 | -0.408 | 1.378 | 0.286 | | | | | | |
| | Birth weight z score | 0.121 | -1.148 | 1.390 | 0.851 | -1.667 | -3.177 | -0.158 | 0.030 | | |

* RSD: residual standard deviation

**Table E7: Univariate and multivariate analysis for time to peak tidal expiratory flow over total expiratory time ($t_{PTEF}/t_E$)**

| | | Univariate Model | | | | Multivariate model | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **Coefficient** | **95% CI** | | **p-value** | **Coefficient** | **95% CI** | | **p-value** | **Adj $R^2$** | **RSD*** |
| $t_{PTEF}/t_E$ % | | | | | | | | | | 1% | 11.974 |
| | Age at study date (weeks) | -0.273 | -1.497 | 0.951 | 0.661 | | | | | | |
| | Male sex | -3.133 | -5.609 | -0.657 | 0.013 | -3.078 | -5.607 | -0.549 | 0.017 | | |
| | Weight-for-age z score | 0.369 | -0.854 | 1.593 | 0.553 | | | | | | |
| | length-for-age z score | -0.251 | -1.151 | 0.649 | 0.584 | | | | | | |
| | Birth weight z score | 0.555 | -0.707 | 1.818 | 0.387 | | | | | | |

* RSD: residual standard deviation

**Table E7a: Univariate analysis, multivariate analysis and reference equation for exhaled nitric oxide, log(eNO)**

| | | Univariate Model | | | | Multivariate model | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Coefficient | 95% CI | | p-value | Coefficient | 95% CI | | p-value | Adj $R^2$ | RSD |
| Log(eNO) | | | | | | | | | | 5% | 0.9175 |
| | Age at study date (weeks) | 0.035 | -0.061 | 0.132 | 0.472 | | | | | | |
| | Male sex | -0.100 | -0.297 | 0.098 | 0.321 | | | | | | |
| | Weight-for-age z score | 0.109 | 0.011 | 0.207 | 0.029 | 0.153 | 0.053 | 0.253 | 0.003 | | |
| | Length-for-age z score | 0.063 | -0.008 | 0.134 | 0.083 | | | | | | |
| | Birth weight z score | 0.129 | 0.029 | 0.229 | 0.012 | | | | | | |
| | Minute ventilation | -0.0006 | -0.0009 | -0.0002 | 0.000 | -0.0006 | -0.0009 | -0.0003 | 0.000 | | |

* RSD: residual standard deviation

**Table E8: Univariate analysis, multivariate analysis and reference equation for exhaled nitric oxide (eNO)**

| | | Univariate Model | | | | Multivariate model | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Coefficient | 95% CI | | p-value | Coefficient | 95% CI | | p-value | Adj $R^2$ | RSD* |
| eNO ppb | | | | | | | | | | 9.5% | 6.544 |
| | Age at study date (weeks) | 0.931 | 0.234 | 1.628 | 0.009 | 1.001 | 0.324 | 1.68 | 0.004 | | |
| | Male sex | -0.339 | 1.776 | 1.097 | 0.643 | | | | | | |
| | Weight-for-age z score | 0.581 | -0.141 | 1.304 | 0.114 | 0.988 | 0.272 | 1.703 | 0.007 | | |
| | Length-for-age z score | 0.315 | -0.207 | 0.837 | 0.236 | | | | | | |
| | Birth weight z score | 0.886 | 0.152 | 1.620 | 0.018 | | | | | | |
| | Minute ventilation | -0.005 | -0.008 | -0.003 | 0.000 | -0.006 | -0.009 | -0.004 | 0.000 | | |

* RSD: residual standard deviation

**Table E9: Univariate and multivariate analysis for nitric oxide output**

| | | Univariate Model | | | | Multivariate model | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **Coefficient** | **95% CI** | | **p-value** | **Coefficient** | **95% CI** | | **p-value** | **Adj $R^2$** | **RSD** |
| NO output ($nL.min^{-1}$) | | | | | | | | | | 3% | 21.033 |
| | Age at study date (weeks) | 2.827 | 0.657 | 4.996 | 0.011 | 2.963 | 0.785 | 5.143 | 0.008 | | |
| | Male sex | -0.784 | -5.255 | 3.686 | 0.730 | | | | | | |
| | Weight-for-age z score | 2.880 | 0.649 | 5.112 | 0.012 | 2.668 | 0.438 | 4.899 | 0.019 | | |
| | Length-for-age z score | 1.077 | -0.545 | 2.699 | 0.192 | | | | | | |
| | Birth weight z score | 2.574 | 0.287 | 4.861 | 0.027 | | | | | | |

* RSD: residual standard deviation

**Table E10: Univariate and multivariate analysis for functional residual capacity (FRC)**

| | | Univariate Model | | | | Multivariate model | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **Coefficient** | **95% CI** | | **p-value** | **Coefficient** | **95% CI** | | **p-value** | **Adj $R^2$** | **RSD*** |
| FRC | (mL) | | | | | | | | | 11% | 16.314 |
| | Age at study date (weeks) | 2.684 | 0.934 | 4.434 | 0.003 | 2.148 | 0.428 | 3.868 | 0.015 | | |
| | Male sex | -3.002 | -6.624 | 0.621 | 0.104 | | | | | | |
| | Weight-for-age z score | 5.035 | 3.314 | 6.756 | 0.000 | 3.638 | 1.579 | 5.697 | 0.001 | | |
| | Length-for-age z score | 2.445 | 1.148 | 3.741 | 0.000 | | | | | | |
| | Birth weight z score | 4.624 | 2.846 | 6.402 | 0.000 | 2.219 | 0.086 | 4.352 | 0.042 | | |

* RSD: residual standard deviation

**Table E11: Univariate analysis for the first moment ratio (M0/M1)**

| | | Univariate Model | | | |
|---|---|---|---|---|---|
| | | Coefficient | 95% CI | | p-value |
| M0/M1 | | | | | |
| | Age at study date (weeks) | 0.008 | -0.005 | 0.022 | 0.227 |
| | Male sex | 0.022 | -0.006 | 0.050 | 0.124 |
| | Weight-for-age z score | 0.006 | -0.008 | 0.019 | 0.397 |
| | Length-for-age z score | 0.003 | -0.007 | 0.013 | 0.510 |
| | Birth weight z score | 0.008 | -0.006 | 0.023 | 0.237 |

**Table E12: Univariate analysis for second moment ratio (M0/M2)**

| | | Univariate Model | | | |
|---|---|---|---|---|---|
| | | Coefficient | 95% CI | | p-value |
| M0/M2 | | | | | |
| | Age at study date (weeks) | 0.068 | -0.478 | 0.183 | 0.250 |
| | Male sex | 0.187 | -0.049 | 0.424 | 0.120 |
| | Weight-for-age z score | 0.047 | -0.068 | 0.163 | 0.420 |
| | length-for-age z score | 0.035 | -0.049 | 0.120 | 0.408 |
| | Birth weight z score | 0.074 | -0.046 | 0.196 | 0.224 |

**Table E13: Univariate analysis for the lung clearance index (LCI)**

| | | Univariate Model | | | |
|---|---|---|---|---|---|
| | | Coefficient | 95% CI | | p-value |
| LCI | (n FRC turnovers) | | | | |
| | Age at study date (weeks) | -0.004 | -0.049 | 0.041 | 0.863 |
| | Male sex | 0.038 | -0.055 | 0.130 | 0.423 |
| | Weight-for-age z score | -0.033 | -0.078 | 0.012 | 0.150 |
| | length-for-age z score | -0.011 | -0.044 | 0.022 | 0.520 |
| | Birth weight z score | -0.004 | -0.051 | 0.043 | 0.857 |

**Table E14: Univariate and multivariate analysis for respiratory system resistance (R)**

| | | Univariate Model | | | | Multivariate model | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **Coefficient** | **95% CI** | | **p-value** | **Coefficient** | **95% CI** | | **p-value** | **Adj $R^2$** | **RSD*** |
| R ($cmH_2O.s.L^{-1}$) | | | | | | | | | | 4% | 15.51 |
| | Age at study date (weeks) | -0.649 | -2.465 | 1.167 | 0.483 | | | | | | |
| | Male sex | 5.131 | 1.553 | 8.709 | 0.005 | 4.918 | 1.292 | 8.544 | 0.008 | | |
| | Weight-for-age z score | 1.164 | -0.613 | 2.941 | 0.198 | 2.994 | 0.731 | 5.257 | 0.010 | | |
| | length-for-age z score | -0.760 | -2.102 | 0.581 | 0.266 | -1.936 | -3.649 | -0.223 | 0.027 | | |
| | Birth weight z score | 0.508 | -1.309 | 2.325 | 0.582 | | | | | | |

* RSD: residual standard deviation

**Table E15: Univariate and multivariate analysis for respiratory system compliance (C)**

| | | Univariate Model | | | | Multivariate model | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **Coefficient** | **95% CI** | | **p-value** | **Coefficient** | **95% CI** | | **p-value** | **Adj $R^2$** | **RSD*** |
| C ($mL.cmH_2O^{-1}$) | | | | | | | | | | 5% | 0.42585 |
| | Age at study date (weeks) | 0.048 | -0.002 | 0.098 | 0.062 | | | | | | |
| | Male sex | -0.177 | -0.276 | -0.078 | 0.000 | -0.160 | -0.257 | -0.058 | 0.002 | | |
| | Weight-for-age z score | 0.039 | -0.010 | 0.088 | 0.120 | | | | | | |
| | Length-for-age z score | 0.054 | 0.018 | 0.091 | 0.004 | 0.049 | 0.010 | 0.012 | 0.085 | | |
| | Birth weight z score | 0.057 | 0.007 | 0.107 | 0.025 | | | | | | |

* RSD: residual standard deviation

**Table E16: Univariate and multivariate analysis for resonant frequency (*fres*)**

| | | Univariate Model | | | | Multivariate model | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **Coefficient** | **95% CI** | | **p-value** | **Coefficient** | **95% CI** | | **p-value** | **Adj $R^2$** | **RSD*** |
| fres (Hz) | | | | | | | | | | 4% | 5.9966 |
| | Age at study date (weeks) | -0.583 | -1.284 | 0.117 | 0.102 | | | | | | |
| | Male sex | 1.944 | 0.557 | 3.331 | 0.006 | 1.659 | 0.257 | 3.061 | 0.020 | | |
| | Weight-for-age z score | -0.981 | -1.663 | -0.299 | 0.005 | | | | | | |
| | Length-for-age z score | -0.795 | -1.308 | -0.282 | 0.003 | -0.738 | -1.258 | -0.222 | 0.005 | | |
| | Birth weight z score | -0.867 | -1.563 | -0.171 | 0.015 | | | | | | |

* RSD: residual standard deviation

**Table E17: Reference equations for tidal breathing, exhaled nitric oxide, multiple breath washout and forced oscillation technique measures in 5-11 week South African infants**

| | Regression equations | Adj $R^2$ | RSD |
|---|---|---|---|
| Tidal breathing | | | |
| Tidal volume | 22.932 + 1.619 * (age weeks) + 2.166 * (male) + 3.185 * (weight for age) | 37% | 5.1 |
| Respiratory rate | 62.859 – 2.103 * (age weeks) – 2.294 * (weight for age) | 7% | 11.5 |
| Minute ventilation | 1,590.33 + 80.993 * (male) + 102.115 * (weight for age) – 41.165* (birth weight z score) | 9% | 294.3 |
| Mean tidal inspiratory flow rate | 59.588 + 4.334 * (Male) + 3.522 * (weight for age) | 15% | 9.8 |
| Mean tidal expiratory flow rate | 49.799 + 2.979 * (weight for age) - 1.667 * (birth weight z score) | 4% | 11.8 |
| Exhaled NO | | | |
| Exhaled nitric oxide | 13.32 + 1.002 * (age weeks) + 0.988 * (weight for age z score) – 0.006 * (minute ventilation) | 9.5% | 6.5 |
| NO output | 12.11 + 2.963 * (age weeks) + 2.668 * (weight age z) | 3% | 21.0 |
| Multiple breath washout | | | |
| FRC | 64.970 + 2.148* (Age at study date) + 3.638* (weight for age z score) + 2.219* (birth weight z score) | 11% | 16.3 |
| **M0/M1, M0/M2 and LCI: see mean data table** | | | |
| Forced oscillation technique | | | |
| Resistance | 45.835 + 4.918 * (male) + 2.994 * (weight for age) – 1.936 * (length for age) | 4% | 15.5 |
| Compliance | 1.06 – 0.160 * (male) + 0.049 * (length for age) | 5% | 0.4 |
| Resonant frequency | 19.933 + 1.779 * (male) – 0.721 * (weight for age) + 1.325 * (Maternal passive smoker) + 3.035*(Maternal active smoker) | 7% | 5.9 |

RSD: residual standard deviation

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Chapter 5

# Determinants of early life lung function in African infants

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## Abstract

**Background:** Low lung function in early life is associated with later respiratory illness. There is limited data on lung function in African infants despite a high prevalence of respiratory disease.

**Aim:** To assess the determinants of early lung function in African infants.

**Method:** Infants enrolled in a birth cohort, the Drakenstein child health study, had lung function measured at 6-10 weeks of age. Measurements, made with the infant breathing via a facemask during natural sleep, included tidal breathing, sulphur hexafluoride multiple breath washout and the forced oscillation technique. Information on antenatal exposures was collected using questionnaires and urine cotinine. Household benzene was measured antenatally.

**Results:** Successful tests were obtained in 645/675 (95%) infants, median (IQR) age of 51 (46-58) days. Infants whose mothers smoked had lower tidal volumes [-1·6mL (95%CI -3·0 to -0.1), p=0.04] and higher lung clearance index [0.1 turnovers (95%CI 0·01 to 0·3), p=0.03] compared to unexposed infants. Infants exposure to alcohol in utero or household benzene had lower time to peak tidal expiratory flow over total expiratory time ratios, 10% (95%CI -15·4 to -3·7), p=0·002) and 3·0% (95%CI -5·2 to -0.7, p=0·01) lower respectively compared to unexposed infants.

**Conclusions:** We identified several factors including maternal smoking, maternal alcohol and household benzene associated with altered early lung function, factors amenable to public health interventions. Long-term study of lung function and respiratory disease in these infants is a priority to develop new strategies to strengthen child health.

## Introduction

Pneumonia is the leading cause of death in children in low and middle income countries (LMIC)[1] and chronic respiratory illness is a common sequela.[2] There is increasing evidence that chronic respiratory disease in later life has its origins in childhood, with low lung function in early life being associated with lung function impairment and chronic respiratory illness in later life.[3] Identifying pre and postnatal factors associated with early lung disease, as measured by lung function, will provide a better understanding of how early life exposures influence childhood lung disease and possibly provide insights into prevention of subsequent chronic respiratory illness. The normal development of the human lung begins *in utero* but continues in post-natal life until adolescence. Thus *in utero* and early life factors that damage or impair lung growth may have a considerable impact on early lung function.

Several risk factors for altered early lung function or respiratory disease in childhood have been identified including infant birth weight[4], prematurity[5], early life respiratory tract infections[6], maternal asthma[7], HIV infection[8], maternal alcohol use[9] and environmental factors such as tobacco smoke exposure[10] and household air pollution[11]. Many of these factors are common in Africa but there are no data on early lung function in these settings despite the high burden of childhood respiratory disease.

We aimed to investigate the impact of antenatal and early life exposures on lung function measured at 6 weeks of age in African infants enrolled in a birth cohort.

## Method

### Setting

Infants enrolled in a birth cohort study, the Drakenstein Child Health study (DCHS)[12], had lung function tested. This study, set in a peri-urban, low socio-economic community in South Africa, aims to investigate the epidemiology and aetiology of childhood respiratory illness and the determinants of child health. Participants were enrolled at two primary care clinics, Mbekweni, serving a predominantly black African population and Newman, serving a predominantly mixed race population. Lung function testing was undertaken at the local hospital.

### Participants

Infants underwent testing at 5 to 11 weeks of age corrected for prematurity (37 weeks). Infants born <32 weeks gestation or with congenital anomalies, were excluded from this analysis. Mothers had spirometric lung function (Jaeger Masterscope, CareFusion, Switzerland) at the same visit, provided they had not had a respiratory infection within the last 2 weeks.

### Exposures

Information regarding antenatal, birth and early life exposures and events were collected by questionnaire at scheduled antenatal and study visits.

Maternal chronic respiratory illness was defined as at least one of: doctor diagnosed asthma, chronic cough or recurrent wheeze in previous 12 months and/or low forced expiratory volume in 1 sec (FEV1), defined as FEV1 < -1.64SD predicted using the Global Lung Initiative multi-ethnic equations.[13] Maternal stress and alcohol intake during pregnancy were assessed using the SRQ-20 and Alcohol, Smoking and Substance Involvement Screening Test (ASSIST) self-reported questionnaires completed at 28 – 32 weeks gestation.[14] The SRQ-20 is a widely used WHO-endorsed measure of psychological distress.[15] We used a dichotomous score of “high risk” versus “low risk”, with “high risk” defined as a score of ≥8.[15] Infants were classified as alcohol-exposed *in utero* if their mother reported daily and/or weekly use of alcohol during three months of pregnancy.

Maternal smoking history was corroborated on a quantitative analysis of maternal urine cotinine (IMMULITE$^{R}$ 1000 Nicotine Metabolite Kit, Siemens Medical Solutions Diagnostics, Glyn Rhonwy, UK) collected antenatally and at birth. Smoking exposure based on urine cotinine was defined as: active smoker if urine cotinine >500 ng.ml$^{-1}$, passive smoker if urine cotinine 10-500 ng.ml$^{-1}$ and non-smoker if urine cotinine <10 ng.ml$^{-1}$.[16] Benzene, a household air pollutant, was measured at an antenatal home visit using a Markes$^{R}$ thermal desorption tube left in the home for two weeks. The South African National Ambient Air Quality standards of 5mcg/m$^{3}$ was used to define above and below threshold values for benzene.[17]

**Infant Lung function measurements**

Lung function measurements included tidal breathing (TBFVL), sulphur hexafluoride (SF6) multiple breath washout (MBW) and the forced oscillation technique (FOT). Infants were tested from July 2012 to Dec 2014 for TBFVL and MBW and, for operational reasons, from October 2012 to Dec 2014 for FOT. Lung function was measured in unsedated infants during quiet sleep and conformed to ATS/ERS guidelines[18 19], as previously published.[20 21]

Tidal breathing measures of tidal volume ($V_T$), respiratory rate and expiratory flow ratios, were collected using the Exhalyser D with ultrasonic flow meter (Ecomedics AG, Duernton, Switzerland) and analyzed using analysis software (Wbreath v3.28.0. Ndd Medizintechnik, AG, Zurich, Switzerland), as described previously.[20] Multiple breath washouts (MBW) measuring the functional residual capacity (FRC) and lung clearance index (LCI) were performed using 4% SF6 as a tracer gas and ultrasonic flow meter (Spirison®, Ecomedics, Duernten, Switzerland) with acquisition and analysis software (Wbreath v3.28.0, Ndd Medizintechnik AG, Zurich, Switzerland) as reported previously. [22] Measurements of respiratory system resistance ($R_{RS}$) and compliance ($C_{RS}$) with the FOT were made with purpose built equipment (University of Szeged, Hungary) using a medium frequency signal, as previously reported.[23 24]

**Ethics**

The study was approved by the Faculty of Health Sciences, Human Research Ethics

Committee, University of Cape Town (401/2009) and by the Western Cape Provincial Health Research Committee. Mothers gave informed, written consent in their first language for their infants to participate.

### Statistical analysis

Descriptive statistics were performed using STATA 13 for windows (STATA Corporation, College Station, Texas, USA). Data are presented as mean and standard deviation (SD) for normally distributed variables and median and interquartile range (IQR) for non-normally distributed variables. Potential determinants of early lung function were identified using a causal diagram. The association of these factors and specific lung function outcomes were investigated using a multiple linear regression model. Co-variates were included in the model if statistically significant at a $p<0{\cdot}05$ or had a biologically plausible bearing on lung function outcomes. Stratification by both enrolment site and sex were assessed, neither were statistically different.

### Results

Between July 2012 and Dec 2014, 690 infants were tested, of whom 15 were excluded as they were very-preterm [13 (2%)] or had congenital abnormalities [2 (0·3%)], figure 1. Infant testing was done at a median (IQR) of 51 (46-58) days, anthropometry of enrolled infants are shown in **table 1**.

Black African infants were of lower socioeconmic status (SES), less likely to have been breastfed and had higher HIV exposure compared to mixed race infants, who had higher rates of tobacco smoke exposure, **table 2**. Despite an HIV prevelance of 18%, no infants were HIV-infected due to a strong prevention of mother to child transmission program that included antiretroviral therapy to all HIV-infected pregnant women and to infants for 6 weeks post partum.

Successful measurements were obtained in 649 (95%) of tidal breathing, 614 (90%) MBW and 508/645 (79%) FOT measures, table 3.

### Associations of early lung function

The results of the univariate and multivariate analyses of associations with lung function measures are shown in **table S1-9**.

### Infant growth and lung maturation

Infant size and age, weight-for-age z score (WAZ), was associated with larger $V_T$, with $V_T$ increasing by 2.5mL for every unit increase in WAZ (p<0·001; 95% CI 1·9-3·1). Birth-weight z score, was associated with lower respiratory rate [difference -3 bpm (95%CI -4 to -1), p<0·001], larger FRC (4mL (1·8 to 6·1), p<0·001] and higher $C_{rs}$, [0·06 mL.cm$H_20^{-1}$ (0·01 to 0·12),p=0·03]. Gestational age was associated with a small but statistically significant decrease in respiratory rate [-1bpm per week increase; (-1·4 to -0·1)p=0·02], increased $V_T$ [0·4mL (0·1 to 0·7)p=0·006,], larger FRC [2·6mL 90·3 to 2·2) p=0·01] and increased $C_{rs}$ [0·03 mL.cm$H_20$ (0·006 to 0·06), p=0·02] at 6-10 weeks of age. However neither somatic growth at birth nor at testing, nor gestational age, were associated with the tidal expiratory flow ratio: time to peak tidal expiratory flow over total expiratory time ($t_{PTEF}/t_E$) and $R_{rs}$.

### Sex and ethnicity

Male infants had larger $V_T$ compared to female infants [2·4mL difference (95%CI 1·4 to 3·4), p<0·001] but lower $t_{PTEF}/t_E$ [-3·3% ( -5·5 to -1·0), p=0·004], higher $R_{rs}$ [4·1 cm-$H_2O$.s.$L^{-1}$ (1·0 to 7·3), p=0.01] and lower $C_{rs}$ [-0·12 mL.cm$H_20$ (-0·2 to -0·03), p=0·008].

Black African infants had a higher respiratory rate [3·4 bpm difference (95%CI 10·8 to 6·0), p=0·01], increased $t_{PTEF}/t_E$ [4·4% (1·7 to 7·2), p=0·002] and increased inspiratory time over total breath time ($t_I/t_{tot}$) [1·3% (0·2 to 2·4), p=0·02], but similar $V_T$ compared to infants of mixed ethnicity. Ethnicity had no effect on other lung function outcomes measured.

### Socioeconmic and environmental factors

Infants' whose mothers smoked during pregnancy had lower $V_T$ [-1·6mL (95%CI -3·0 to -0·1), p=0·04] and higher LCI [0.1 FRC turnovers (0·01 to 0·3), p=0·03] compared to infants whose mothers did not smoke during pregancy. The $t_{PTEF}/t_E$ , FRC and $C_{rs}$ were

also lower in smoke exposed compared to non-exposed infants, but these did not remain statistically signficant after correcting for predictors and confounders. Infants living in homes with high levels of household benzene had lower $t_{PTEF}/t_E$ [-3·0 % (-5·2 to -0·7), p=0·01] compared to infants from low level households. Household benzene exposure had no effect on other lung function outcomes. Socioeconmic status was not associated with lung function differences.

**Maternal factors**

High maternal distress scores, breastfeeding practises and history of maternal chronic respiratory illness were not associated with lung function outcomes. Infants of HIV-infected mothers had higher $V_T$ [1.7 mL (95%CI 0·06 to 3·3), p=0·04] compared to infants who were not HIV-exposed, but no other differences in lung function were observed.

Infants whose mothers drank alcohol during pregnancy had lower respiratory rates [-7 bpm (-13 to -2), p=0.009 ] higher tider volumes [3·0 mL (CI 0·4 to 5·5), p=0·2] and lower $t_{PTEF}/t_E$ [-10% (-15·4 to -3·7), p=0·002 ] compared to infants whose mothers did not drink alcohol.

**Previous pneumonia**

Thirty-five (5%) infants had a lower respiratory tract infection prior to lung function testing (**table 2**). These infants had similar lung function at 6 weeks to infants who had not had pneumonia after adjusting for confounding factors (**tables S1-S9**).

## Dicussion

This is the first study from a low middle income country setting to show several antenatal and early life factors that impact on lung function, affecting both lung structure and control of breathing in 6 week old African infants. Known factors such as infant growth, sex and maternal smoking were identified as well as novel risk factors such as maternal alcohol ingestion and HIV-exposure.

Different antenatal and early life exposures affected either lung structural development as evidenced by reduced lung volumes, ventilation homogeneity and lung compliance;

or control of breathing with effects on respiratory rate and flow ratios or both. Somatic growth, age and gestational age primarily affected lung development, while ethnicity, alcohol exposure, HIV exposure or benzene affected control of breathing; sex and tobacco smoke exposure affected both.

As expected infant size both at testing and at birth was associated with increased lung size and respiratory system compliance ($C_{rs}$) at 6 weeks. Size at birth is associated with early lung developement.[25] The fact that resistance was not associated with somatic growth in this study is likely related to the measurement method which, by using a mask over the mouth and nose, includes the resistance of the upper airway which is large and dominates the measured effect on the respiratory system resistance.[26]

Our findings of sex differences in lung function are consistent with previous reports of low lung function in male infants[27 28], suggesting that male infants may have a less mature respiratory system at birth with smaller airways and FRC compared to female infants. Lower early lung function in boys may be one factor contributing to the higher rate of pneumonia in boys.[29]

The effect of ethnic differences may be difficult to distinguish from sociocultural and environmental factors. However even after adjusting for exposure differences, lung function remained different between Black African and mixed race infants. Black African infants had higher respiratory rate and longer flow ratios, but similar lung volumes and impedance to mixed race infants, suggesting a different early pattern of breathing in African infants. This is consistent with previously described ethnic differences between Black Afro-Caribbean and Caucasian European infants .[27 30]

Smoke exposed infants had smaller lung volumes and reduced ventilation efficiency suggesting early structural lung impairment. Previous studies have shown that both *in utero* smoke exposure [30] and early life environmental exposure to tobacco smoke (ETS) impair early lung function.[10] In this study we were unable to distinguish between the effect on lung function of *in utero* or early life smoke exposure as lung function was measured a few weeks after birth. As our cohort had an extremely high passive smoke exposure rate (75%), it is likely that some of the effects seen are due to ETS in early life. Although

altered control of breathing has been described in smoke exposed infants,[31] our lung function measures may not be sensitive nor specific enough to detect these effects.

Infants exposed to high household benzene levels had lower $t_{PTEF}/t_E$ which may be related to airway obstruction or to breathing pattern. The $t_I/t_{TOT}$ more specifically reflects the central control of breathing, but was not associated with benzene exposure in this study. Further research evaluating the impact of antenatal and early exposure to indoor air pollutants on lung health is needed.

Socioeconmic status did not impact lung function outcomes. This may reflect the overall pooor socio-economic status of the populations studied. Prior pneumonia also did not affect lung function, however the number of children with pneumonia was very small, and the study was therefore not adequately powered to investigate this.

Consistent with several other studies, we found no association between maternal chronic respiratory illness and infant lung function. [32-34]

This is the first study to show an effect of alcohol exposure on infant lung function. The deleterious effect of alcohol exposure on the developing fetal brain is well understood and described, providing a possible basis for altered control of breathing in alcohol exposed infants. In addition *in utero* alcohol exposure reduces surfactant protein and alveolar macrophage function and increases lung susceptibility to trauma and infection in animal models.[35 36] Animal studies have also shown alcohol exposure during pregnancy to impact lung growth and structure[9], although some do not persist into early postnatal life.[37] The number of alcohol exposed infants included was small and may have been an underestimate of infants exposed due to under-reporting. However, this represents the minimum number of alcohol exposed infants, with a very strong association for impaired lung function demonstrated. Further research of the potential mechanisms and impact is needed.

This is also the first study to show an association between HIV exposure and lung function, with exposed infants having higher $V_T$, suggesting an effect on control of breathing. This effect may be mediated through HIV or due to ART taken by mothers and infants

with dysregulation of metablic pathways. Further research is needed including long term followup of infants, which we are currently undertaking.

Strengths of this study include the large sample size, the high success rate of testing, the reproducibility of lung function testing, the comprehensive lung function testing that was done and the measurement of many, diverse potential risk factors that may impact on lung function. In addition, this is the first study to provide lung function measurements in African infants. A limitation is the possible lack of generalisability to other LMICs or to other ethnic groups. However, many of the risk factors described are highly prevalent in LMICs.

We identified several risk factors for impaired lung function that are amenable to public health interventions and several that require further study. Avoidance of maternal smoking and smoking cessation program for pregnant women should be a priority, avoidance of alcohol should be stressed and implementing strategies to reduce exposure to benzene should be undertaken in this population. Long term study of lung function and respiratory health in these infants is a priority that we are undertaking to inform new strategies to strengthen child health in LMICs.

**Figure 1: Description of cohort**

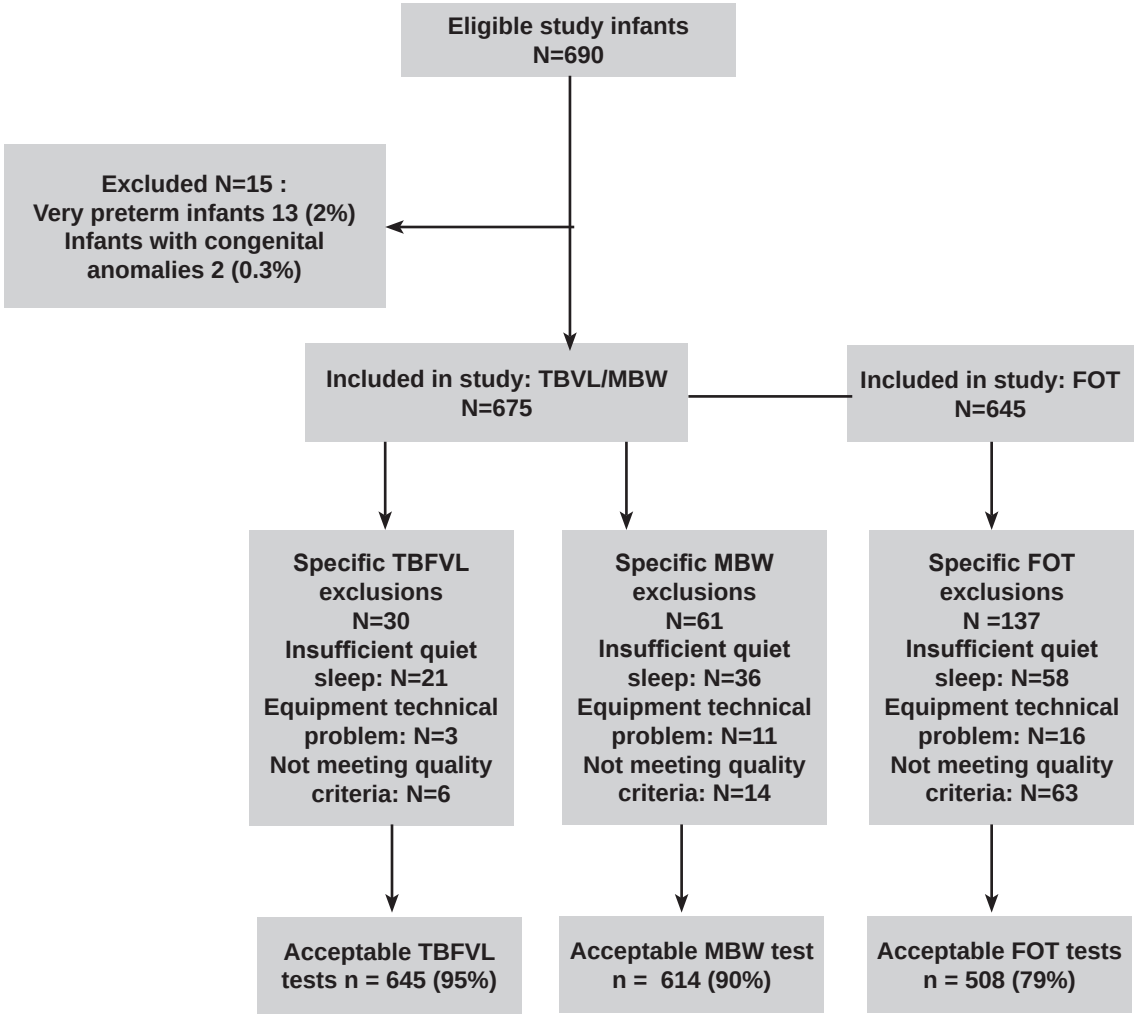


**Table 1: Anthropometry by enrolment site**

| | Mbekweni (African Black ethnicity) N=329 | Newman (African mixed race ethnicity) N=346 | Total N=675 | P |
|---|---|---|---|---|
| | **Med (25; 75)** | **Med (25; 75)** | **Med (25; 75)** | |
| Age in days | 50 (46; 57) | 52 (47; 59) | 51 (46; 58) | 0·1 |
| Weight kg | 4.9 (4.4; 5.5) | 4·7 (4·2; 0·3) | 4·9 (4·3; 5·4) | 0·004 |
| Z score weight | -0.2 (-0.8; 0.6) | -0·6 (-1·4; 0·1) | -0·4 (-1·2; 0·4) | 0·0000 |
| Length cm | 56 (53; 57) | 55 (53; 57) | 55 (53; 57) | 0·08 |
| Z score length | -0·7(-1·7; 0·3) | -1·0 (-2·0; 0·0) | -0·8 (-1·8; 0·1) | 0·003 |
| Birth weight kg | 3·1 (2·8; 3·4) | 3·0 (2·6; 3·4) | 3·0 (2·7; 3·4) | 0·02 |
| Z score birth weight | -0·4(-1·3; 0·3) | -0·8 (-1·5; -0·1) | -0·6 (-1·4; 0·1) | 0·0003 |
| Gestation weeks | 39 (38; 40) | 39 (38; 40) | 39 (38; 40) | 0·7 |

**Table 2: Demographics and socio-economic factors by enrolment site**

| | Mbekweni (African black) N=294 | Newman (African mixed race) N=335 | Total N=629 | P |
|---|---|---|---|---|
| | N (%) | N (%) | N (%) | |
| **Sex** male | 138 (47) | 194 (58) | 332 (53) | 0·01 |
| **Maternal smoking** | | | | |
| Non-smoker | 92 (31) | 37 (11) | 129 (21) | 0·000 |
| Passive exposure | 148 (50) | 125 (37) | 273 (43) | |
| Smoker | 42 (14) | 162 (48) | 204 (32) | |
| Unknown | | | 23 (4) | |
| **Household air pollution** | | | | |
| High benzene | 135 (46) | 131 (39) | 266 (42) | 0·1 |
| **Socio-economic Status** | | | | |
| Low | 115 (39) | 62 (19) | 177 (28) | 0·000 |
| Low-moderate | 77 (26) | 76 (23) | 153 (24) | |
| Moderate-high | 55 (19) | 84 (25) | 139 (22) | |
| High | 47 (16) | 112 (33) | 159 (25) | |
| **Breastfeeding** | | | | |
| Exclusive | 120 (41) | 153 (46) | 273 (43) | 0·000 |
| Mixed | 110 (37) | 172 (51) | 282 (45) | |
| None | 64 (22) | 10 (3) | 74 (12) | |
| **Maternal chronic respiratory illness** | 22 (8) | 29 (9) | 51 (8) | 0·6 |
| **Maternal HIV infected** | 107 (36) | 9 (0.3) | 116 (18) | 0·000 |
| **Maternal stress high** | 60 (20) | 71 (21) | 131 (21 | 0·9 |
| **Antenatal alcohol exposure** | 6 (2) | 19 (6) | 25 (4) | 0·07 |
| **Previous pneumonia** | 12 (4) | 23 (7) | 35 (5) | 0·08 |

**Table 3: Lung function outcomes**

| Lung function variable | Mean (SD) | Median | IQR |
|---|---|---|---|
| Tidal breathing n=649 | | | |
| Respiratory rate $n.min^{-1}$ | 49 (11·5) | 47 | 41; 55 |
| Tidal volume mL | 34·7 (6·3) | 34·2 | 30·0; 38·6 |
| $t_{PTEF}/t_E$ * % | 38·7 (12·2) | 38·6 | 30·2; 46·2 |
| $t_I/t_{TOT}$ * % | 45·3 (4·8) | 45·2 | 42·0; 48·8 |
| $t_E/t_{TOT}$ * % | 54·7 (4·8) | 54·8 | 51·2; 58·0 |
| **Multiple breath washout n=614** | | | |
| Functional residual capacity (FRC) mL | 77·0 (15·9) | 74·5 | 65·5; 86·3 |
| Lung clearance index n turnovers | 7·2 (0·4) | 7·1 | 6·9; 7·4 |
| **Forced oscillation technique n=508** | | | |
| Resistance ($R_{rs}$) $cmH_2O.s.L^{-1}$ | 47·9 (15·3) | 45·0 | 37·5; 55·8 |
| Compliance ($C_{RS}$) $mL.cmH_2O^{-1}$ | 0·94 (0·4) | 0·88 | 0·67; 1·14 |

* $t_{PTEF}/t_E$: time to peak tidal expiratory flow over total expiratory time; $t_I/t_{TOT}$: inspiratory time over total breath time; $t_E/t_{TOT}$: expiratory time over total breath time

## Supplementary information

### Table S1: Univariate and multivariate analysis of respiratory rate

| Univariate (n=654) | | | | Multivariate model (n=459) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Coefficient | 95% CI | | p-value | Coefficient | 95% CI | | p-value | Adj $R^2$ | Residual Std Dev |
| **Respiratory rate (n.min$^{-1}$)** | | | | | | | | | 7% | 10.94 |
| **Infant growth and lung maturation** | | | | | | | | | | |
| **Weight-for-age z score** | -1.621 | -2.420 | -0.822 | <0.001 | 0.347 | -0.962 | 1.656 | 0.603 | | |
| **Gestation** | -0.653 | -1.091 | -0.215 | 0.004 | -0.949 | -1.596 | -0.302 | 0.004 | | |
| **Birth weight z score** | -1.937 | -2.775 | -1.099 | <0.001 | -2.717 | -4.120 | -1.314 | <0.001 | | |
| **Gender and ethnicity** | | | | | | | | | | |
| **Male gender** | 0.381 | -1.424 | 2.186 | 0.679 | -1.109 | -3.190 | 0.972 | 0.295 | | |
| **Ethnicity Black African** | 2.764 | 0.974 | 4.555 | 0.003 | 3.385 | 0.822 | 5.948 | 0.010 | | |
| **Environmental and socio-economic factors** | | | | | | | | | | |
| **Smoking: Non smoker** | | | | | | | | | | |
| **Passive** | -1.910 | -4.288 | 0.468 | 0.115 | -0.622 | -3.294 | 2.050 | 0.647 | | |
| **Active** | -0.418 | -2.936 | 2.099 | 0.744 | 1.869 | -1.307 | 5.045 | 0.248 | | |
| **Benzene** | -1.025 | -3.072 | 1.021 | 0.325 | -1.779 | -3.832 | 0.275 | 0.089 | | |
| **Social Economic Status: Highest quartile** | | | | | | | | | | |
| **Lowest quartile** | 0.375 | -2.121 | 2.872 | 0.768 | | | | | | |
| **Low-moderate quartile** | 1.380 | -1.198 | 3.958 | 0.294 | | | | | | |
| **Moderate-high quartile** | 0.058 | -2.571 | 2.687 | 0.965 | | | | | | |
| **Maternal factors** | | | | | | | | | | |
| **Maternal stress** | -1.050 | -3.302 | 1.202 | 0.360 | | | | | | |
| **Feeding choice: Exclusive breastfeeding** | | | | | | | | | | |
| **Mixed feeding** | -0.116 | -2.035 | 1.803 | 0.906 | | | | | | |
| **No breastfeeding** | 0.390 | -2.581 | 3.361 | 0.797 | | | | | | |
| **Maternal chronic respiratory illness** | -0.674 | -3.381 | 2.033 | 0.625 | | | | | | |
| **Maternal HIV infection** | 0.699 | -1.621 | 3.020 | 0.554 | -0.214 | -3.165 | 2.737 | 0.887 | | |
| **Maternal alcohol intake during pregnancy** | -2.692 | -7.102 | 1.718 | 0.231 | -7.199 | -12.627 | -1.771 | 0.009 | | |
| **Previous pneumonia** | 2.698 | -1.301 | 6.697 | 0.186 | 2.797 | -1.762 | 7.356 | 0.229 | | |

**Table S2: Univariate and multivariate analysis of tidal volume**

| Univariate (n=654) | | | | Multivariate model (n=459) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Coefficient | 95% CI | | p-value | Coefficient | 95% CI | | p-value | Adj $R^2$ | Residual Std Dev |
| Tidal volume (mL) | | | | | | | | | 33% | 5.14 |
| Infant growth and lung maturation | | | | | | | | | | |
| Weight-for-age z score | 2.863 | 2.487 | 3.239 | <0.001 | 2.492 | 1.876 | 3.109 | <0.001 | | |
| Gestation | 0.670 | 0.438 | 0.902 | <0.001 | 0.454 | 0.149 | 0.759 | 0.004 | | |
| Birth weight z score | 1.803 | 1.368 | 2.239 | <0.001 | 0.492 | -0.171 | 1.155 | 0.146 | | |
| Gender and ethnicity | | | | | | | | | | |
| Male gender | 1.323 | 0.356 | 2.289 | 0.007 | 2.416 | 1.438 | 3.395 | <0.001 | | |
| Ethnicity | 1.076 | 0.109 | 2.043 | 0.029 | -0.804 | -2.011 | 0.403 | 0.191 | | |
| Environmental and socio-economic factors | | | | | | | | | | |
| Smoking: Non smoker | | | | | | | | | | |
| Passive | -0.170 | -1.446 | 1.107 | 0.794 | -0.553 | -1.811 | 0.705 | 0.388 | | |
| Active | -2.491 | -3.842 | -1.139 | <0.001 | -1.549 | -3.045 | -0.053 | 0.042 | | |
| Benzene | 0.467 | -0.648 | 1.583 | 0.411 | 0.803 | -0.168 | 1.775 | 0.105 | | |
| Social Economic Status: highest quartile | | | | | | | | | | |
| Lowest quartile | -1.059 | -2.401 | 0.283 | 0.122 | | | | | | |
| Low-moderate quartile | -0.070 | -1.456 | 1.316 | 0.921 | | | | | | |
| Moderate-high quartile | 0.171 | -1.242 | 1.584 | 0.812 | | | | | | |
| Maternal factors | | | | | | | | | | |
| Maternal stress | -0.599 | -1.800 | 0.603 | 0.328 | | | | | | |
| Feeding choice: Exclusive breastfeeding | | | | | | | | | | |
| Mixed feeding | 0.577 | -0.452 | 1.606 | 0.271 | 0.747 | -0.279 | 1.773 | 0.153 | | |
| No breastfeeding | 1.746 | 0.153 | 3.339 | 0.032 | 1.499 | -0.351 | 3.349 | 0.112 | | |
| Maternal chronic respiratory illness | 0.256 | -1.201 | 1.713 | 0.731 | | | | | | |
| Maternal HIV infection | 1.686 | 0.442 | 2.929 | 0.008 | 1.679 | 0.059 | 3.300 | 0.042 | | |
| Maternal alcohol | -1.118 | -3.472 | 1.237 | 0.352 | 2.955 | 0.403 | 5.506 | 0.023 | | |
| Previous pneumonia | -2.033 | -4.182 | 0.117 | 0.064 | -1.025 | -3.170 | 1.120 | 0.348 | | |

**Table S3: Univariate and multivariate analysis of the ratio of time to peak tidal expiratory flow over total expiratory time (tPTEF/tE)**

| Univariate (n=654) | | | | Multivariate model (n=459) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Coefficient | 95% CI | | p-value | Coefficient | 95% CI | | p-value | Adj $R^2$ | Residual Std Dev |
| $t_{PTEF}/t_E$ (%) | | | | | | | | | 10% | 11.87 |
| **Infant growth and lung maturation** | | | | | | | | | | |
| Weight-for-age z score | 0.023 | -0.833 | 0.880 | 0.957 | -0.913 | -2.327 | 0.500 | 0.205 | | |
| Gestation | 0.379 | -0.087 | 0.845 | 0.111 | 0.287 | -0.412 | 0.987 | 0.420 | | |
| Birth weight z score | -0.021 | -0.922 | 0.880 | 0.964 | 0.602 | -0.920 | 2.123 | 0.437 | | |
| **Gender and ethnicity** | | | | | | | | | | |
| Male gender | -2.750 | -4.650 | -0.850 | 0.005 | -3.196 | -5.440 | -0.952 | 0.005 | | |
| Ethnicity | 3.971 | 2.087 | 5.854 | <0.001 | 4.414 | 1.648 | 7.179 | 0.002 | | |
| **Environmental and socio-economic factors** | | | | | | | | | | |
| Smoking: Non smoker | | | | | | | | | | |
| Passive | -2.014 | -4.543 | 0.514 | 0.118 | -0.127 | -3.012 | 2.758 | 0.931 | | |
| Active | -3.139 | -5.815 | -0.462 | 0.022 | -0.036 | -3.465 | 3.393 | 0.984 | | |
| Benzene | -1.706 | -3.893 | 0.482 | 0.126 | -2.931 | -5.158 | -0.704 | 0.010 | | |
| Social Economic Status: highest quartile | | | | | | | | | | |
| Lowest quartile | 0.764 | -1.881 | 3.410 | 0.571 | | | | | | |
| Low-moderate quartile | -0.364 | -3.095 | 2.367 | 0.794 | | | | | | |
| Moderate-high quartile | 0.399 | -2.387 | 3.184 | 0.779 | | | | | | |
| **Maternal factors** | | | | | | | | | | |
| Maternal stress | 0.774 | -1.629 | 3.177 | 0.527 | | | | | | |
| Feeding choice: Exclusive breastfeeding | | | | | | | | | | |
| Mixed feeding | -2.322 | -4.333 | -0.311 | 0.024 | -2.192 | -4.544 | 0.160 | 0.068 | | |
| No breastfeeding | 3.018 | -0.096 | 6.132 | 0.057 | 1.086 | -3.158 | 5.330 | 0.615 | | |
| Maternal chronic respiratory illness | -2.419 | -5.278 | 0.441 | 0.097 | -2.422 | -5.816 | 0.973 | 0.162 | | |
| Maternal HIV infection | 3.218 | 0.773 | 5.664 | 0.010 | -1.503 | -5.232 | 2.225 | 0.429 | | |
| Maternal alcohol | -6.201 | -10.879 | -1.523 | 0.009 | -8.485 | -14.359 | -2.612 | 0.005 | | |
| Previous pneumonia | -3.984 | -8.211 | 0.243 | 0.065 | -1.647 | -6.569 | 3.275 | 0.511 | | |

**Table S4: Univariate and multivariate analysis of the ratio of inspiratory time over total breath time ($t_I/t_{TOT}$)**

| Univariate (n=654) | | | | | Multivariate model (n=457) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Coefficient** | **95% CI** | | **p-value** | **Coefficient** | **95% CI** | | **p-value** | **Adj $R^2$** | **Residual Std Dev** |
| $t_I/t_{TOT}$ (%) | | | | | | | | | 3% | 4.60 |
| **Infant growth and lung maturation** | | | | | | | | | | |
| **Weight-for-age z score** | -0.245 | -0.575 | 0.085 | 0.145 | -0.016 | -0.564 | 0.531 | 0.954 | | |
| **Gestation** | -0.150 | -0.329 | 0.029 | 0.100 | -0.086 | -0.357 | 0.184 | 0.532 | | |
| **Birth weight z score** | -0.293 | -0.636 | 0.051 | 0.095 | -0.351 | -0.938 | 0.237 | 0.241 | | |
| **Gender and ethnicity** | | | | | | | | | | |
| **Male gender** | -0.436 | -1.167 | 0.295 | 0.242 | -0.542 | -1.412 | 0.329 | 0.222 | | |
| **Ethnicity** | 1.697 | 0.977 | 2.416 | <0.001 | 1.275 | 0.203 | 2.348 | 0.020 | | |
| **Environmental and socio-economic factors** | | | | | | | | | | |
| **Smoking: Non smoker** | | | | | | | | | | |
| **Passive** | -0.437 | -1.392 | 0.517 | 0.369 | -0.106 | -1.224 | 1.012 | 0.852 | | |
| **Active** | -0.852 | -1.863 | 0.159 | 0.098 | -0.260 | -1.589 | 1.069 | 0.701 | | |
| **Benzene** | 0.049 | -0.778 | 0.875 | 0.908 | -0.213 | -1.072 | 0.647 | 0.627 | | |
| **Social Economic Status: highest quartile** | | | | | | | | | | |
| **Lowest quartile** | 0.356 | -0.651 | 1.364 | 0.488 | | | | | | |
| **Low-moderate quartile** | 0.045 | -1.001 | 1.091 | 0.933 | | | | | | |
| **Moderate-high quartile** | 0.587 | -0.478 | 1.653 | 0.279 | | | | | | |
| **Maternal factors** | | | | | | | | | | |
| **Maternal stress** | -0.634 | -1.561 | 0.294 | 0.180 | | | | | | |
| **Feeding choice: Exclusive** | | | | | | | | | | |
| **Mixed feeding** | -0.089 | -0.866 | 0.687 | 0.822 | | | | | | |
| **None** | 0.090 | -1.126 | 1.306 | 0.885 | | | | | | |
| **Maternal chronic illness** | -0.540 | -1.640 | 0.560 | 0.336 | | | | | | |
| **Maternal HIV infection** | 0.434 | -0.507 | 1.374 | 0.366 | -0.389 | -1.623 | 0.846 | 0.537 | | |
| **Maternal alcohol** | -0.565 | -2.348 | 1.218 | 0.534 | -1.499 | -3.770 | 0.773 | 0.195 | | |
| **Previous pneumonia** | 0.167 | -1.462 | 1.796 | 0.841 | 0.964 | -0.943 | 2.872 | 0.321 | | |

**Table S5: Univariate (n=654) and multivariate (n=459) analysis of the ratio of expiratory time over total breath time ($t_E/t_{TOT}$)**

| Univariate (n=654) | | | | | Multivariate (n=459) model | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Coefficient | 95% CI | | p-value | Coefficient | 95% CI | | p-value | Adj $R^2$ | Residual Std Dev |
| $t_E/t_{TOT}$ (%) | | | | | | | | | 3% | 4.620 |
| **Infant growth and lung maturation** | | | | | | | | | | |
| **Weight-for-age z score** | 0.245 | -0.085 | 0.575 | 0.145 | 0.016 | -0.531 | 0.564 | 0.954 | | |
| **Gestation** | 0.150 | -0.029 | 0.329 | 0.100 | 0.086 | -0.184 | 0.357 | 0.532 | | |
| **Birth weight z score** | 0.293 | -0.051 | 0.636 | 0.095 | 0.351 | -0.237 | 0.938 | 0.241 | | |
| **Gender and ethnicity** | | | | | | | | | | |
| **Male gender** | 0.436 | -0.295 | 1.167 | 0.242 | 0.542 | -0.329 | 1.412 | 0.222 | | |
| **Ethnicity** | -1.697 | -2.416 | -0.977 | <0.001 | -1.275 | -2.348 | -0.203 | 0.020 | | |
| **Environment and socio-economic factors** | | | | | | | | | | |
| **Smoking: Non smoker** | | | | | | | | | | |
| **Passive** | 0.437 | -0.517 | 1.392 | 0.369 | 0.106 | -1.012 | 1.224 | 0.852 | | |
| **Active** | 0.852 | -0.159 | 1.863 | 0.098 | 0.260 | -1.069 | 1.589 | 0.701 | | |
| **Benzene** | -0.049 | -0.875 | 0.778 | 0.908 | 0.213 | -0.647 | 1.072 | 0.627 | | |
| **Social Economic Status: highest quartile 4** | | | | | | | | | | |
| **Lowest quartile** | -0.356 | -1.364 | 0.651 | 0.488 | | | | | | |
| **Low-moderate quartile** | -0.045 | -1.091 | 1.001 | 0.933 | | | | | | |
| **Moderate-high quartile** | -0.587 | -1.653 | 0.478 | 0.279 | | | | | | |
| **Maternal factors** | | | | | | | | | | |
| **Maternal stress** | 0.634 | -0.294 | 1.561 | 0.180 | | | | | | |
| **Feeding choice: Exclusive** | | | | | | | | | | |
| **Mixed feeding** | 0.089 | -0.687 | 0.866 | 0.822 | | | | | | |
| **None** | -0.090 | -1.306 | 1.126 | 0.885 | | | | | | |
| **Maternal chronic illness** | 0.540 | -0.560 | 1.640 | 0.336 | | | | | | |
| **Maternal HIV infection** | -0.434 | -1.374 | 0.507 | 0.366 | 0.389 | -0.846 | 1.623 | 0.537 | | |
| **Maternal alcohol** | 0.565 | -1.218 | 2.348 | 0.534 | 1.499 | -0.773 | 3.770 | 0.195 | | |
| **Previous pneumonia** | -0.167 | -1.796 | 1.462 | 0.841 | -0.964 | -2.872 | 0.943 | 0.321 | | |

**Table S6: Univariate and multivariate (n=435) analysis of the functional residual capacity (FRC)**

| Univariate (n=563) | | | | Multivariate model (n=435) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Coefficient | 95% CI | | p-value | Coefficient | 95% CI | | p-value | Adj $R^2$ | Residual Std Dev |
| FRC (mL) | | | | | | | | | 10% | 15.90 |
| **Infant growth and lung maturation** | | | | | | | | | | |
| **Weight-for-age z score** | 3.645 | 2.526 | 4.765 | <0.001 | 0.228 | -1.731 | 2.187 | 0.819 | | |
| **Gestation** | 1.205 | 0.593 | 1.817 | <0.001 | 1.308 | 0.350 | 2.266 | 0.008 | | |
| **Birth weight z score** | 2.981 | 1.802 | 4.160 | <0.001 | 3.965 | 1.839 | 6.091 | <0.001 | | |
| **Gender and ethnicity** | | | | | | | | | | |
| **Male gender** | -2.338 | -4.880 | 0.205 | 0.071 | -0.194 | -3.323 | 2.936 | 0.903 | | |
| **Ethnicity** | 2.710 | 0.173 | 5.248 | 0.036 | 2.764 | -1.214 | 6.742 | 0.173 | | |
| **Environmental and socio-economic factors** | | | | | | | | | | |
| **Smoking: Non smoker** | | | | | | | | | | |
| **Passive** | -1.987 | -5.355 | 1.381 | 0.247 | -1.995 | -6.037 | 2.046 | 0.332 | | |
| **Active** | -4.676 | -8.228 | -1.124 | 0.010 | -2.684 | -7.521 | 2.154 | 0.276 | | |
| **Benzene** | -0.517 | -3.516 | 2.482 | 0.735 | -0.436 | -3.517 | 2.645 | 0.781 | | |
| **Social Economic Status: highest quartile** | | | | | | | | | | |
| **Lowest quartile** | -3.658 | -7.177 | -0.138 | 0.042 | -2.540 | -7.138 | 2.057 | 0.278 | | |
| **Low-moderate quartile** | -2.770 | -6.401 | 0.861 | 0.135 | -0.696 | -5.169 | 3.777 | 0.760 | | |
| **Moderate-high quartile** | -3.726 | -7.420 | -0.031 | 0.048 | -2.925 | -7.403 | 1.553 | 0.200 | | |
| **Maternal factors** | | | | | | | | | | |
| **Maternal stress** | -1.365 | -4.571 | 1.841 | 0.403 | | | | | | |
| **Feeding choice: Exclusive** | | | | | | | | | | |
| **Mixed feeding** | -0.027 | -2.736 | 2.681 | 0.984 | | | | | | |
| **None** | 2.736 | -1.435 | 6.907 | 0.198 | | | | | | |
| **Maternal chronic respiratory illness** | 0.670 | -3.144 | 4.484 | 0.730 | | | | | | |
| **Maternal HIV infection** | 2.415 | -0.833 | 5.663 | 0.145 | 0.556 | -3.850 | 4.962 | 0.804 | | |
| **Maternal alcohol** | 1.012 | -5.345 | 7.369 | 0.755 | 5.298 | -2.869 | 13.466 | 0.203 | | |
| **Previous pneumonia** | -3.207 | -8.867 | 2.453 | 0.266 | 0.269 | -6.674 | 7.212 | 0.939 | | |

**Table S7: Univariate and multivariate analysis of the lung clearance index (LCI)**

| Univariate (n=563) | | | | Multivariate model (n=435) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Coefficient | 95% CI | | p-value | Coefficient | 95% CI | | p-value | Adj $R^2$ | Residual Std Dev |
| LCI (n FRC turnovers) | | | | | | | | | 4% | 0.44 |
| **Infant growth and lung function** | | | | | | | | | | |
| **Weight-for-age z score** | -0.012 | -0.044 | 0.020 | 0.456 | -0.015 | -0.069 | 0.039 | 0.585 | | |
| **Gestation** | -0.007 | -0.024 | 0.010 | 0.404 | 0.006 | -0.021 | 0.032 | 0.667 | | |
| **Birth weight z score** | 0.006 | -0.027 | 0.039 | 0.739 | 0.013 | -0.045 | 0.071 | 0.664 | | |
| **Gender and ethnicity** | | | | | | | | | | |
| **Male gender** | 0.035 | -0.035 | 0.105 | 0.324 | 0.069 | -0.016 | 0.154 | 0.111 | | |
| **Ethnicity** | 0.007 | -0.062 | 0.077 | 0.837 | 0.085 | -0.020 | 0.189 | 0.114 | | |
| **Environmental and socio-economic factors** | | | | | | | | | | |
| **Smoking: Non smoker** | | | | | | | | | | |
| **Passive** | 0.060 | -0.031 | 0.151 | 0.196 | 0.057 | -0.052 | 0.166 | 0.304 | | |
| **Active** | 0.113 | 0.017 | 0.209 | 0.021 | 0.139 | 0.011 | 0.268 | 0.034 | | |
| **Benzene** | 0.071 | -0.011 | 0.152 | 0.088 | 0.054 | -0.031 | 0.138 | 0.213 | | |
| **Social Economic Status: Highest quartile** | | | | | | | | | | |
| **Lowest quartile** | 0.000 | -0.097 | 0.096 | 0.996 | | | | | | |
| **Low-moderate quartile** | 0.035 | -0.065 | 0.135 | 0.491 | | | | | | |
| **Moderate-high quartile** | 0.029 | -0.073 | 0.130 | 0.579 | | | | | | |
| **Maternal factors** | | | | | | | | | | |
| **Maternal stress** | 0.057 | -0.030 | 0.145 | 0.198 | | | | | | |
| **Feeding choice: Exclusive breastfeeding** | | | | | | | | | | |
| **Mixed feeding** | -0.023 | -0.097 | 0.051 | 0.540 | | | | | | |
| **No breastfeeding** | -0.012 | -0.126 | 0.103 | 0.840 | | | | | | |
| **Maternal chronic respiratory illness** | 0.011 | -0.093 | 0.116 | 0.832 | | | | | | |
| **Maternal HIV infection** | -0.005 | -0.094 | 0.084 | 0.908 | -0.031 | -0.151 | 0.090 | 0.614 | | |
| **Maternal alcohol** | -0.093 | -0.266 | 0.080 | 0.290 | -0.222 | -0.446 | 0.002 | 0.052 | | |
| **Previous pneumonia** | 0.105 | -0.050 | 0.260 | 0.182 | 0.120 | -0.070 | 0.310 | 0.214 | | |

**Table S8: Univariate and multivariate analysis of resistance ($R_{RS}$)**

| Univariate (n=568) | | | | Multivariate model (n=393) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Coefficient | 95% CI | | p-value | Coefficient | 95% CI | | p-value | Adj $R^2$ | Residual Std Dev |
| $R_{RS}$ $cmH_2O.L.s^{-1}$ | | | | | | | | | 4% | 15.33 |
| **Infant growth and lung maturation** | | | | | | | | | | |
| **Weight-for-age z score** | 0.077 | -1.059 | 1.212 | 0.895 | 0.858 | -1.115 | 2.832 | 0.393 | | |
| **Gestation** | -0.391 | -1.021 | 0.239 | 0.224 | -0.778 | -1.787 | 0.231 | 0.130 | | |
| **Birth weight z score** | -0.778 | -1.964 | 0.407 | 0.198 | -1.537 | -3.644 | 0.571 | 0.152 | | |
| **Gender and ethnicity** | | | | | | | | | | |
| **Male gender** | 3.491 | 0.963 | 6.020 | 0.007 | 4.137 | 1.005 | 7.270 | 0.010 | | |
| **Ethnicity** | -0.820 | -3.368 | 1.728 | 0.528 | -0.952 | -4.821 | 2.918 | 0.629 | | |
| **Environmental and socio-economic factors** | | | | | | | | | | |
| **Smoking: Non smoker** | | | | | | | | | | |
| **Passive** | 0.583 | -2.847 | 4.012 | 0.739 | -1.549 | -5.626 | 2.528 | 0.456 | | |
| **Active** | -0.372 | -3.971 | 3.227 | 0.839 | -4.716 | -9.533 | 0.100 | 0.055 | | |
| **Benzene** | -0.019 | -3.013 | 2.976 | 0.990 | 0.412 | -2.676 | 3.500 | 0.793 | | |
| **Social Economic Status: highest quartile** | | | | | | | | | | |
| **Lowest quartile** | 0.175 | -3.342 | 3.692 | 0.922 | | | | | | |
| **Low-moderate quartile** | -1.444 | -5.063 | 2.175 | 0.434 | | | | | | |
| **Moderate-high quartile** | -0.033 | -3.757 | 3.690 | 0.986 | | | | | | |
| **Maternal factors** | | | | | | | | | | |
| **Maternal stress** | -0.592 | -3.748 | 2.564 | 0.713 | | | | | | |
| **Feeding choice: Exclusive breastfeeding** | | | | | | | | | | |
| **Mixed feeding** | -2.151 | -4.833 | 0.532 | 0.116 | | | | | | |
| **No breastfeeding** | -2.731 | -7.111 | 1.649 | 0.221 | | | | | | |
| **Maternal chronic illness** | 3.117 | -0.652 | 6.886 | 0.105 | | | | | | |
| **Maternal HIV infection** | -1.853 | -5.125 | 1.420 | 0.267 | -1.356 | -5.833 | 3.121 | 0.552 | | |
| **Maternal alcohol** | 0.300 | -5.825 | 6.424 | 0.923 | 2.098 | -5.710 | 9.905 | 0.598 | | |
| **Previous pneumonia** | 1.211 | -4.873 | 7.295 | 0.696 | -3.459 | -10.992 | 4.075 | 0.367 | | |

**Table S9: Univariate and multivariate analysis of tidal compliance ($C_{RS}$)**

| Univariate (n=568) | | | | | Multivariate model (n=393) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Coefficient** | **95% CI** | | **p-value** | **Coefficient** | **95% CI** | | **p-value** | **Adj $R^2$** | **Residual Std Dev** |
| $C_{RS}$ ($mL.cmH_20^{-1}$) | | | | | | | | | 4% | 0.42 |
| **Infant growth and lung maturation** | | | | | | | | | | |
| **Weight-for-age z score** | 0.037 | 0.008 | 0.066 | 0.014 | -0.0254 | -0.0791 | 0.0282 | 0.352 | | |
| **Gestation** | 0.029 | 0.013 | 0.046 | <0.001 | 0.0332 | 0.0058 | 0.0605 | 0.017 | | |
| **Birth weight z score** | 0.033 | 0.002 | 0.064 | 0.037 | 0.0620 | 0.0047 | 0.1194 | 0.034 | | |
| **Gender and ethnicity** | | | | | | | | | | |
| **Male gender** | -0.101 | -0.166 | -0.036 | 0.003 | -0.1162 | -0.2018 | -0.0305 | 0.008 | | |
| **Ethnicity** | 0.046 | -0.020 | 0.112 | 0.169 | -0.0258 | -0.1361 | 0.0844 | 0.645 | | |
| **Environmental and socio-economic factor** | | | | | | | | | | |
| **Smoking: Non smoker** | | | | | | | | | | |
| **Passive** | -0.030 | -0.119 | 0.059 | 0.504 | 0.0108 | -0.1024 | 0.1240 | 0.852 | | |
| **Active** | -0.117 | -0.210 | -0.024 | 0.014 | -0.0692 | -0.2037 | 0.0653 | 0.312 | | |
| **Benzene** | 0.076 | -0.004 | 0.156 | 0.063 | 0.0679 | -0.0163 | 0.1522 | 0.114 | | |
| **Social Economic Status: Highest quartile** | | | | | | | | | | |
| **Lowest quartile** | -0.092 | -0.182 | -0.001 | 0.048 | -0.0727 | -0.2002 | 0.0547 | 0.263 | | |
| **Low-moderate quartile** | -0.049 | -0.143 | 0.044 | 0.298 | -0.0344 | -0.1545 | 0.0857 | 0.574 | | |
| **Moderate-high quartile** | -0.005 | -0.101 | 0.091 | 0.918 | 0.0310 | -0.0924 | 0.1543 | 0.622 | | |
| **Maternal factors** | | | | | | | | | | |
| **Maternal stress** | -0.026 | -0.108 | 0.056 | 0.534 | | | | | | |
| **Feeding choice: Exclusive breastfeeding** | | | | | | | | | | |
| **Mixed feeding** | -0.002 | -0.072 | 0.067 | 0.948 | | | | | | |
| **No breastfeeding** | 0.074 | -0.040 | 0.187 | 0.204 | | | | | | |
| **Maternal chronic respiratory illness** | -0.022 | -0.120 | 0.075 | 0.651 | | | | | | |
| **Maternal HIV infection** | 0.054 | -0.031 | 0.138 | 0.213 | 0.0570 | -0.0650 | 0.1790 | 0.359 | | |
| **Maternal alcohol** | -0.096 | -0.255 | 0.063 | 0.235 | -0.0834 | -0.2955 | 0.1286 | 0.440 | | |
| **Previous pneumonia** | -0.212 | -0.369 | -0.056 | 0.008 | -0.0951 | -0.3001 | 0.1099 | 0.362 | | |

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Chapter 6

# Lung function in the first year of life in African infants: effect of lower respiratory tract infections

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## Abstract

### Rationale

Lower respiratory tract infections are a major cause of childhood morbidity and mortality. It is unknown whether infants are predisposed to respiratory infections due to impaired lung function or whether infections reduce lung function. We aimed to investigate the impact of early life exposures, including lower respiratory tract infections on lung function during infancy.

### Methods

Infants enrolled in an African birth cohort study had lung function at six weeks and one year. Testing, during quiet natural sleep, included tidal breathing, exhaled nitric oxide and multiple breath washout measures. Risk factors for impaired lung health were collected longitudinally. Respiratory tract infection surveillance was performed and episodes investigated.

### Results

Lung function was tested in 304 children. Seventy-two (23%) infants had lower respiratory tract infections during the first year of life. Lower respiratory tract infections were independently associated with decreased tidal volume (-3.6 mL, 95%CI -6.6 to -0.5; p=0·02) and increased respiratory rate (6%, 95%CI 1.01 to 1.10; p=0·01), with stronger effect if the infant was hospitalized. Repeat episodes of respiratory infections further increased respiratory rate (5%, 95%CI 1.02 to 1.08; p=0.001), decreased tidal volume (-2.5 mL, 95%CI -4·7 to -0·3; p=0·02) and increased the lung clearance index (0·13 turnovers, 95%CI 0·00 to 0·26; p=0·05).

### Conclusions

Early life lower respiratory tract infection reduces lung function at one year, independent of baseline lung function. Preventing early life lower respiratory tract infections is important to optimise lung function and promote respiratory health in childhood.

*Word count:* 249

*Keywords:* pediatrics, pulmonary function test, epidemiology

## Introduction:

Respiratory disease is a leading cause of childhood mortality and morbidity globally, particularly in low and middle-income countries (LMIC).[1 2] Amongst these, lower respiratory tract infections (LRTI) are the leading cause of mortality, with the peak incidence in the first year of life.[1] Despite this the impact of LRTI on lung growth and development has not been well studied, especially in LMIC where the burden of LRTI is particularly high. Lung development and maturation is incomplete at birth and continues during the first years of life making lungs particularly vulnerable to damage during this critical time of lung development. Low lung function in early life has been shown to track through childhood and into adulthood[3 4]; further, children born with low lung function have an increased risk of respiratory infections and wheezing illnesses in childhood.[5-10] LRTI in infancy has been associated with reduced lung function in later childhood and adulthood.[11-13] However, the sparse data from longitudinal studies of lung function in high income settings suggest that the effect of respiratory tract infections on later lung function may be mediated through reduced lung function prior to the infective episode. [9 10] Understanding the importance of LRTI and other risk factors on lung development and subsequent risk of respiratory illness, particularly in LMIC where disease burden is concentrated, may inform strategies for optimising lung health and preventing respiratory disease.

We aimed to investigate lung function longitudinally from six weeks to one year of age and the impact of early life exposures, including LRTI on lung growth during the one year in an African birth cohort study, the Drakenstein Child Health Study (DCHS).

## Methods:

### Study design and participants

Infants enrolled in the DCHS, were followed from birth until a year of age, during which lung function testing was done and close investigation of any LRTI episode was undertaken. The DCHS is a birth cohort study situated in a peri-urban, low socio-economic area outside Cape Town in South Africa.[14] Mothers were enrolled antenatally and followed through pregnancy at one of two primary care clinics; mother-child pairs were followed from birth. Infants attended scheduled study visits at six, ten, fourteen weeks and six, nine and twelve months of age, with lung function assessed at six weeks and one year.

Household tobacco smoke exposure or maternal smoking information was collected by study questionnaire at each study visit. Maternal alcohol intake during pregnancy was assessed using the Alcohol, Smoking and Substance Involvement Screening Test (ASSIST) self-reported questionnaires completed at 28 – 32 weeks gestation.[15] Infants were classified as being alcohol-exposed in utero if their mother reported daily or weekly use of alcohol during the past three months.

A strong LRTI surveillance system was established as part of the DCHS and detailed investigation was done at any episode of LRTI. WHO clinical case definitions were used to define an episode of LRTI (cough or difficulty breathing and increased respiratory rate or lower chest wall in-drawing in a child> two months) or severe LRTI (child < two months with increased respiratory rate or lower chest wall in-drawing, or any general danger sign in a child of any age).[16] In addition each suspected case was examined by study staff to confirm the presence of lower respiratory disease. LRTI surveillance was established through community health workers, use of cell phones, a dedicated study contact person available 24 hours and a network of study staff at community based sites.[17]

The study was approved by the Faculty of Health Sciences, Human Research Ethics Committee, University of Cape Town (401/2009; 423/2012) and by the Western Cape Provincial Health Research Committee. Mothers gave informed, written consent in their first language for their infants to participate.

### Lung function measures

Lung function testing was undertaken first at six (5-11) weeks of age corrected for prematurity (<37 weeks) and then at one year (11-13 months). All testing was done in unsedated, behaviourally assessed quiet sleep as previously described.[18 19] Testing included measures of tidal breathing (tidal volume, respiratory rate, inspiratory and expiratory flow ratios), exhaled nitric oxide (eNO) a measure of eosinophilic airway inflammation and sulphur-hexafluoride (SF6) multiple breath washout (MBW), measuring functional residual capacity (FRC) and lung clearance index (LCI), a measure of ventilation distribution. Tidal breathing and eNO measures were collected using the Exhalyser D with ultrasonic flow meter (Ecomedics AG, Duernton, Switzerland) and analysed using analysis software (Wbreath v3.28.0. Ndd Medizintechnik, AG, Zurich, Switzerland) and the MBW measures were performed using 4% SF6 as a tracer gas and ultrasonic flow meter (Spiroson®, Ecomedics, Duernten, Switzerland) with acquisition and analysis software (Wbreath v3.28.0, Ndd Medizintechnik AG, Zurich, Switzerland), as previously described.[18 19] Low lung function at six weeks was defined as lung function outcome below the lower limit of normal using reference equations as published.[19]

### Statistical analysis

Lung function outcome measurements were modelled using multiple linear regression to assess the impact of different early life exposures on lung function attained at one year. Base models were first constructed for each lung function outcome separately using Directed Acyclic Graphs (DAGs) for confounder selection. DAGs minimal adjust set of variables were selected using a step by step approach described here.[20] Early life exposures of interest were then added to the base models one at a time and assessed individually. The only exception to this were infants' lung function measurements at baseline (6 weeks) and weight measured at 1 year which were included in most base models because they explained large percentage of the variability of lung function at 1 year. Height and weight were not included in any models simultaneously because of high correlation between them (sample correlation = 0.87).

Interactions were first explored between each early life exposure of interest and lung function at 6 weeks as well as weight at 1 year and nine were found. Interactions be-

tween weight for age, height for age, age at test, sex and lung function at six weeks as well as non-linear age terms were also explored based on the previous literature.[21-23] Interactions were retained in the overall model if Wald test p-values were less than 0.1.

Estimated coefficients, 95% confidence intervals and p-values were recorded for each early life exposure of interest (Table 3, supplement S2-7). Confounders and interactions included in the overall models are summarized in table S1.

Infants were then stratified into quartile by overall model's predicted lung function at 1 year, excluding early life exposure of interest. Odds ratios were calculated for each early life exposure based on the direction of effect of each lung function outcome: either the upper quartile was compared to the lower quartile (for 1 sided effects) or upper and lower quartiles were compared to the middle quartile separately (for 2 sided effects). Estimated odds ratios, 95% confidence intervals and p-values were recorded for each early life exposure in the on line supplement.

Statistical analyses were performed using STATA 13 for windows (STATS Corporation, College Station, Texas, USA). Weight (WAZ) and height (HAZ) for age z scores were calculated using the WHO Child Growth Standards "I grow up" STATA package.[24]

**Results**

Of 1152 infants enrolled at birth in the DCHS between July 2012 and March 2015, 856 infants had reached six weeks of which 741 (87%) had lung function tested at six weeks; 496 infants had reached one year of which 398 (80%) were tested at a mean (SD) age of 12.0 (0.7) months, figure 1. Paired unsedated six week and one year lung function was completed in 304 children, of which 279 (70%) TBFVL, 266 (67%) eNO and 262 (66%) MBW tests were successful with good quality results. Reasons for exclusions or failed tests are shown in figure 1. The anthropometry and results of lung function at six weeks and one year, with average within-subject interval change is shown in table 1. Between the six week and one year test the Respiratory rate decreased by an average of 17 b.p.m, tidal volume ($V_T$) increased by an average of 56.1 mL, a 2.6 fold increase and the FRC by 120 mL, a 2.5 fold increase. The $t_{PTEF}/t_E$ decreased 8% and the average lung clearance index reduced by 0.4 turnovers.

The cohort demographics and exposures are shown in table 2. Two hundred and one (66%) infants were exposed to household tobacco, with 98 (32%) infants having a mother who smoked. Twelve (4%) infants had mothers who drank during pregnancy. Sixty-one (21%) mothers were HIV-infected, however due to the well-functioning prevention of mother to child transmission programme no infants were HIV infected. There were 72 (24%) infants who had at least one episode of LRTI in the first year, of which 38/72 (53%) had associated wheeze. Fifteen (5%) infants had more than one episode. Nine infants (3%) had severe LRTI, 24 (8%) infants were hospitalized and 9 (3%) received supplemental oxygen.

**Lung function between six weeks and one year**

Figure 2 illustrates the tracking of lung function from six weeks to one year. Infants with low lung function at six weeks had 8% higher respiratory rate (95%CI 1.04 to 1.12; p<001), 8.6 mL lower $V_T$ (95%CI -12.3 to -5.0; p<0.001), 4% lower $t_{PTEF}/t_E$ and 13mL lower FRC (95%CI -25.6 to -0.1; p=0.05) at one year compared to infants who did not have low baseline function.

**Determinants of lung function at one year**

The results of the linear regression model of associations with lung function at one year are summarised in table 3 and full analyses are shown in table S2 to 7. After controlling for low baseline lung function, growth and other confounding factors (table S1), infants who had a LRTI episode had reduced lung function at one year compared to infants who did not have a LRTI during the first year of life, with an average 6% higher respiratory rate (95% CI 1.01 to 1.10; p=0.01) and 3.6 mL lower $V_T$ (95%CI -6.6 to -0.54; p=0.02). Timing of a LRTI was not associated with lung function at one year, but infants with more than one episode of LRTI had a higher LCI compared to infants who did not have a LRTI (0.13 turnover higher, 95%CI 0·00 to 0·26; p=0·05). The effect on one year lung function was greater if the LRTI required hospitalization with a 10% increase in respiratory rate (95%CI 1·03 to 1·17; p=0·007) and 6 mL decrease in $V_T$ (95%CI -11·2 to -0·7; p=0·03). Low baseline lung function was not associated with risk of LRTI during the first year of life, but low baseline lung function and LRTI had an additive effect on lung function outcome at one year. Exhaled nitric oxide was not associated with LRTI or any lung function

outcome at one year, table S7 and S13.

Infant growth was a strong predictor of lung function at one year. For every WAZ increase infants had a 4.2 mL higher $V_T$ (95%CI 3.0 to 5.3; p<0.001), 13.3 mL higher FRC (95%CI 6.8 to 19.8; p<0.001) and 0.13 turnover lower LCI (95%CI -0.21 to -0.06; p<0.001). Age related weight gain measured as change in WAZ between six week and one year testing, though associated with increased $V_T$ and FRC, was associated with a 4% lower $t_{PTEF}/t_E$ for every WAZ increase (95%CI 0.92 to 1.0; p=0.03).

Exposure to household tobacco smoke during the first year of life was associated with a higher LCI [0.2 turnovers (95%CI 0.00 to 0.39), p=0.046] compared to infants who were unexposed. Infant's whose mothers were HIV-infected had a 6% increase in respiratory rate (95%CI 1.01 to 1.11; p=0.028) at one year compared to infants whose mothers were uninfected.

Neither being born premature nor maternal alcohol intake during pregnancy where significantly associated with lung function change during the first year after adjusting for confounding factors.

**Independent predictors of low lung function at one year**

The results of the regression analysis of infant growth and early life exposures on the risk of having low lung function at one year are expressed as odds ratios (OR), 95% CI and p values summarised in table 3, with comprehensive output shown in table S8 -13 in the online supplement.

LRTI in the first year of life more than doubled a child's risk of having a respiratory rate in the highest quartile at one year [OR 2·2 (95%CI 1·1 to 4·0), p=0·01] and every additional LRTI increased a child's risk of having an increased respiratory rate at 1 year by 50% [OR 1·5 (CI 1·0 to 2·3) p=0·05].

Improved nutrition during the first year of life as measured by a change in WAZ between six weeks and one year predicted better lung function at one year. Every unit increase in WAZ reduced the chance of having a low $V_T$ at one year by 91% [OR 0.09 (CI 0.04 to

0.2), p<0.001], low FRC by 53% [OR 0.47 (95%CI 0.3 to 0.7), p<0.001] and a high LCI by 92% [OR 0.08 (CI 0.04 to 0.2), p<0.001]. Similarly an increase in HAZ reduced the risk of having low $V_T$ by 34% [OR 0.66 (CI 0.5 to 0.9, p=0.003) and a high LCI by 50% [OR 0.5 (CI 0.4 to 0.7), p<0.001].

Late preterm infants (33-37 weeks gestation) had nearly three times the risk [OR 2.6 (95%CI 1.2 to 5.7), p=0.002] of having a low $V_T$ at one year compared to infants born at term. There were too few very preterm infants (<33 weeks gestation) in each quartile to assess risk. Infants of HIV-infected mothers were 58% less likely to have low $V_T$ [OR 0.42 (95%CI 0.18 to 0.98) p=0·3] and 70% less likely to have low FRC [OR 0.3 (0.11 to 0·83) p=0.02] at one year compared to infants whose mothers were uninfected. They were also three times more likely to have a $t_{PTEF}/t_E$ in the highest quartile at one year [OR 3.0 (95%CI 1.3 to 7.0), p=0.01]. Infants whose mother drank alcohol during pregnancy were five times more like to have a reduced FRC [OR 4.9 (95%CI 1.2 to 20.2) p=0.03].

Being exposed to household tobacco smoke doubled an infant's risk of being in the lowest quartile $V_T$ at one year [OR 1.9 (95%CI 1.0 to 3.8), p=0.06]. This effect was similar if the infant's mother was the household smoker [OR 1.88 (95%CI 1.0 to 3.8), p=0.065].

**Discussion**

This is the first study in a LMIC to describe changes in lung function and the impact of early life LRTI on child lung health. Lung function during this critical period of lung development tracked from early life, with low lung function soon after birth a strong predictor of lung function at one year. LRTI were identified as a key factor associated with reduced lung function at one year, with more severe disease and recurrent infections associated with a further reduction in lung function. Other factors identified that impacted normal lung growth included infant nutrition, prematurity, exposure to household tobacco and maternal HIV infection.

Previous reports of LRTI in early life and later lung function suggested that the effect of LRTI on lung function was mediated by pre-existing low lung function.[10 25] In the current study low lung function at six weeks was not associated with increased risk of LRTI during the first year. The independent effect of LRTI and infection severity on lung func-

tion outcomes at one year is an important finding in view of the fact that lung function is known to track to later life and increase risk of later respiratory disease.[3 4] Longitudinal follow-up of these infants is currently underway.

The tracking of lung function during the first year of life is in keeping with described patterns of early lung development. [26 27] The increase in $V_T$ and FRC during the first year reflects the rapid and non-linear association of lung and somatic growth[28] and the decrease of $t_{PTEF}/t_E$ , the dysanaptic growth of lung parenchyma and airways during this period of development.[29]

Although postnatal growth is associated with increased lung volumes, large postnatal weight gain is associated with reduced expiratory flows.[30 31] We found decreased expiratory flow ratios ($t_{PTEF}/t_E$) in infants who had increased WAZ between six weeks and one year despite this being associated with increased lung volumes. Hence optimising nutrition is important in promoting respiratory health in infancy.

We have already described the negative impact of maternal smoking during pregnancy on lung function at 6 weeks in this cohort.[32 33] Subsequent household exposure to tobacco smoke had an effect, independent of baseline lung function, of increased LCI and a trend to a higher FRC. This may reflect airway inflammation and/or damage rather than impaired growth. The fact that maternal smoking did not have a stronger effect than household smoke exposure reflects the very high exposure to environmental smoke and overcrowded living conditions in this setting.

Although prematurity was not independently associated with lung function outcomes at one year, being born premature was a strong predictor of having $V_T$ in the lowest quartile, suggesting that decreased lung volumes in preterm infants are mediated by reduced somatic size.[34] Most children in this cohort were born full term and those that were preterm were late preterm infants, hence the impact of prematurity on lung function is unlikely to be substantial in this cohort.

Infants whose mothers were HIV-infected had higher respiratory rates compared to those with uninfected mothers, equating to a significantly raised minute ventilation in HIV

exposed compared to HIV unexposed infants [2·8 $L.min^{-1}$ vs. 2·6 $L.min^{-1}$ (95%CI 2·6 to 2·7); p<0·001]. This may be related to a sustained effect of exposure to the HIV virus in utero, exposure to antiretroviral therapy as part of prevention of disease transmission or differing nutritional management in early life. The effect of maternal HIV and early life antiretroviral therapy on respiratory control in early life needs further study.

Strengths of this study are the large sample size of matched lung function collected by the same investigators, using the same testing techniques. Lung function was undertaken in a community-based cohort with robust LRTI surveillance and simultaneous measurement of comprehensive risk factors for impaired lung growth. Our symptom-based definition of LRTI allowed us to assess the impact on lung function at a population level of community acquired respiratory tract infections. This definition aligns with relevant epidemiological studies investigating LRTI in high burden settings.[35] A further strength is measurement of lung function prior to an infective episode, enabling accurate assessment of pre-respiratory tract infection lung health. Limitations of the study are the potential lack of generalizability to other settings with different exposures.

In summary, this study describes the tracking of lung function in the first year of life in a cohort of infants living in a high respiratory disease burden setting. It identifies LRTI as an independent risk factor for reduced lung function at one year, highlighting the importance of development of strategies to prevent LRTI in early life. Other risk factors include infant nutrition, maternal HIV infection and smoke exposure, factors amenable to public health interventions. Given that infant lung function tracks into later life and plays a role in chronic respiratory disease, preventing respiratory illness in young children, reducing exposure to environmental tobacco smoke and optimising nutrition are further key priorities to strengthen child respiratory health.

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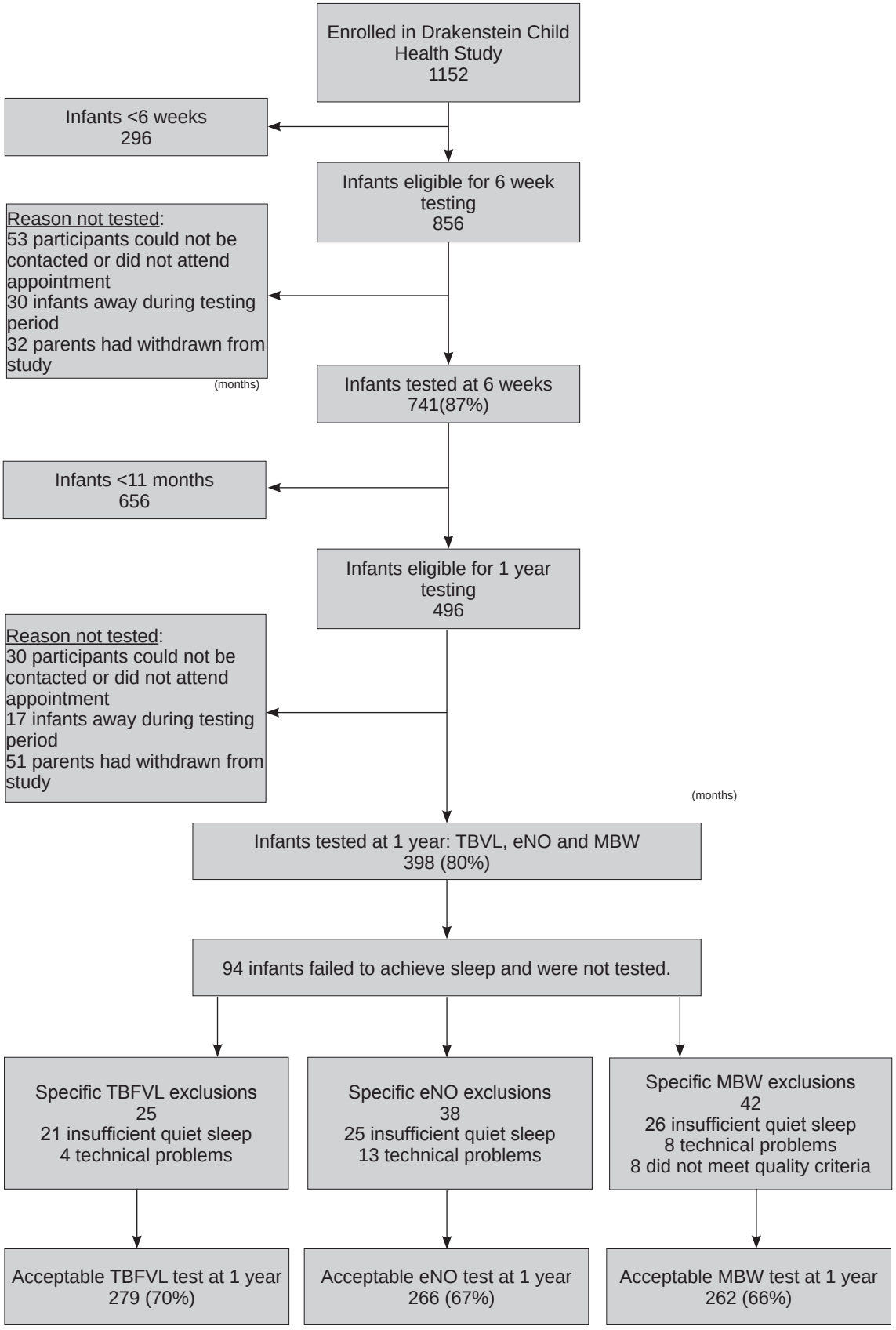
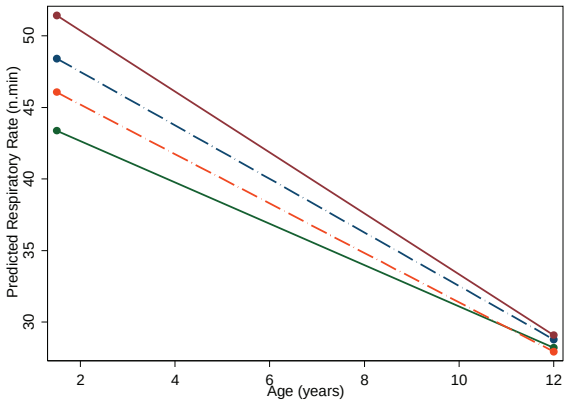
**Figure 1. Cohort description before exclusions (N=1152)**



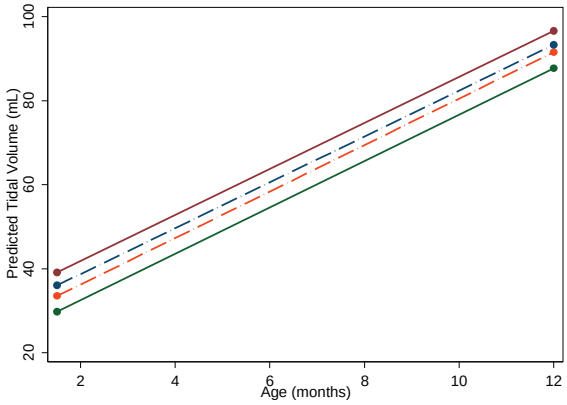

TBFVL: tidal breathing measures; eNO: exhaled nitric oxide; MBW: SF6 multiple breath washout

**Figure 2: Lung function outcomes in quartiles at 6 weeks and 1 year.**

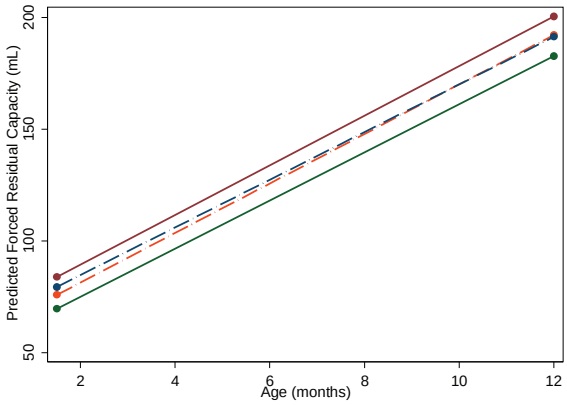
**A.** Respiratory rate (breaths per minute)




**B.** Tidal volume (mL)




**C.** Functional residual capacity (mL)




**D.** Ratio of peak tidal expiratory flow over total expiratory time

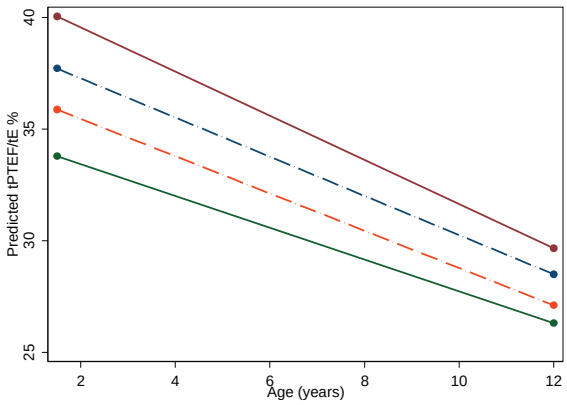



**Table 1 Infant anthropometry and lung function outcomes at six weeks and one year and within-subject interval change**

| | 6 week test mean (SD) | 1 year test mean (SD) | Within-subject interval change mean (SE) n=304 |
|---|---|---|---|
| **Age** months | 1.72 (0.30) | 11.97 (0.70) | 10.25 (0.03) |
| **Weight** kg | 4.88 (0.76) | 9.22 (1.40) | 4.41 (0.07) |
| **Weight for age** Z score | -0.39 (1.11) | -0.11 (1.31) | 0.29 (0.08) |
| **Height** cm | 55.07 (3.13) | 73.33 (3.18) | 18.27 (0.21) |
| **Height for age** Z score | -0.88 (1.45) | -0.63 (1.30) | 0.25 (0.10) |
| **Tidal breathing measures n=279** | | | |
| **Respiratory rate** $n.min^{-1}$ | 48.81 (11.59) | 29.43 (4.92) | -17.06 (11.99) |
| **Tidal volume** mL | 34.65 (6.36) | 90.45 (13.30) | 56.08 (12.00) |
| $\mathbf{t_{PTEF}/t_E}$ % | 38.90 (12.40) | 30.83 (11.61) | -7.98 (13.27) |
| $\mathbf{t_I/t_{TOT}}$ % | 45.33 (4.71) | 43.94 (4.45) | -1.56 (5.34) |
| **Exhaled nitric oxide n=266** | | | |
| Exhaled NO | 9.8 (6.5) | 9.4 (9.2) | -0.4 (0.6) |
| **Multiple breath washout measures n=262** | | | |
| **Functional residual capacity** mL | 77.39 (16.24) | 196.51(45.73) | 119.92 (22.50) |
| **Lung clearance index** n turnovers | 7.16 (0.44) | 6.73 (0.67) | -0.39 (0.84) |

$t_{PTEF}/t_E$: time to peak tidal expiratory flow over total expiratory time, $t_I/t_{TOT}$: inspiratory time over total breath time

**Table 2 Demographics of infants tested at one year of age (n=304)**

| | N (%) |
|---|---|
| Male sex | 157 (51·6) |
| Black African ethnicity | 138 (45·4) |
| Socio-economic status[1] | |
| Highest quartile | 67 (22·0) |
| Moderate-high quartile | 112 (36·8) |
| Low-moderate quartile | 54 (17·8) |
| Lowest quartile | 71 (23·4) |
| Preterm | |
| < 37 weeks gestation | 45 (14·8) |
| <32 weeks gestation | 6 (2·0) |
| Small for gestational age | 25 (8·2) |
| Lower respiratory tract infection (LRTI) | |
| LRTI episode | 72 (23·7) |
| >1 LRTI | 15 (4·9) |
| LRTI with wheeze | 38 (12·5) |
| Hospitalized LRTI | 24 (7·9) |
| LRTI requiring oxygen | 9 (3·0) |
| Severe LRTI[2] | 9 (3·0) |
| Household tobacco smoke exposure (excluding mother) N=303 | 201 (66·3) |
| Maternal smoking n=303 | 98 (32·3) |
| Maternal alcohol use in pregnancy (n=280) | 12 (4·3) |
| Maternal HIV infection[3] | 61 (20·1) |

[1]Quartiles are internal comparisons within a low socio-economic cohort; [2]Severe LRTI was diagnosed in children younger than 2 months with tachypnoea (>60 breaths per min) or lower chest wall indrawing, or in children of any age if the child had a general danger sign; [3]No infants were HIV infected

**Table 3 Associations of lung function and predictors of lowest quartile lung function outcomes at one year of age**

| | Overall model (95% CI) | Lowest predicted lung function quartile OR (95% CI) |
|---|---|---|
| **Baseline low lung function** | | |
| Respiratory rate % change # | 1.08 (1.04, 112) | 2.12 (1.2, 3.7) |
| Tidal volume mL | -8.64 (-12.29, -4.97) | 5.33 (2.12, 13.38) |
| $t_{PTEF}/t_E$ % change # | 0.76 (0.55, 0.77) | N/A |
| Functional residual capacity mL | -12.81 (-25.58, -0.05) | 0.83 (0.76, 0.91) |
| Lung clearance index | 0.05 (-0.14, 0.25) | 0.81 (0.36, 1.82) |
| **Lower respiratory tract infection** | | |
| Respiratory rate % change | 1.06 (1.01, 1.10) | 2.17 (1.16, 4.04) |
| Tidal volume mL (raw score) | -3.57 (-6.6, -0.54) | 1.22 (0.60, 2.51) |
| $t_{PTEF}/t_E$ % (log transformed) | 1.07 (0.98, 1.16) | 1.52 (0.76, 2.98) |
| Functional residual capacity mL | -9.05 (-21.29, 3.20) | 0.76 (0.37, 1.58) |
| Lung clearance index | 0.13 (-0.06, 0.33) | 0.98 (0.47, 2.07) |
| **Infant growth- change in WAZ** | | |
| Respiratory rate % change # | 1.00 (0.98, 1.02) | 1.06 (0.82, 1.36) |
| Tidal volume mL (raw score) | 3.26 (1.97, 4.56) | 0.09 (0.04, 0.20) |
| $t_{PTEF}/t_E$ % (log transformed) | 0.96 (0.92, 1.00) | 1.03 (0.79, 1.35) |
| Functional residual capacity mL | 11.67 (6.32, 17.01) | 0.47 (0.34, 0.66) |
| Lung clearance index | -0.09 (-0.17, 0.00) | 0.08 (0.04, 0.19) |
| **Household smoke** | | |
| Respiratory rate % change # | 1.00 (0.96, 1.04) | 0.86 (0.49, 1.49) |
| Tidal volume mL (raw score) | 0.49 (-2.50, 3.48) | 1.90 (0.97, 3.75) |
| $t_{PTEF}/t_E$ % (log transformed) | 0.99 (0.92, 1.08) | 1.44 (0.75, 2.76) |
| Functional residual capacity mL | 11.00 (-0.26, 22.27) | 1.83 (0.93, 3.63) |
| Lung clearance index | 0.19 (0.00, 0.39) | 1.07 (0.53, 2.17) |
| **Prematurity (<37 weeks)** | | |
| Respiratory rate % change # | 0.94 (0.88, 1.00) | 2.01 (0.95, 4.27) |
| Tidal volume mL (raw score) | -0.22 (-4.83, 4.38) | 2.56 (1.15, 5.73) |
| $t_{PTEF}/t_E$ % (log transformed) | 0.98 (0.84, 1.11) | 1.18 (0.53, 2.63) |
| Functional residual capacity mL | -12.70 (-32.20, 6.81) | 1.21 (0.52, 2.82) |
| Lung clearance index | 0.04 (-0.22, 0.31) | 0.88 (0.39, 2.01) |
| **Maternal HIV** | | |
| Respiratory rate % change # | 1.06 (1.01, 1.11) | 1.66 (0.88, 3.13) |
| Tidal volume mL (raw score) | 0.70 (-3.41, 4.82) | 0.42 (0.18, 0.98) |
| $t_{PTEF}/t_E$ % (log transformed) | 1.05 (0.95, 1.15) | 0.66 (0.28, 1.55) |
| Functional residual capacity mL | 0.29 (-12.10, 12.67) | 0.30 (0.11, 0.83) |
| Lung clearance index | 0.18 (-0.06, 0.41) | 0.81 (0.36, 1.83) |
| **Maternal alcohol** | | |
| Respiratory rate % change # | 0.99 (0.96, 1.03) | 0.64 (0.12, 3.37) |
| Tidal volume mL (raw score) | -1.04 (-8.40, 6.33) | 7.55 (0.90, 63.14) |
| $t_{PTEF}/t_E$ % (log transformed) | 1.02 (0.81, 1.22) | 3.27 (0.89, 2.04) |
| Functional residual capacity mL | -2.68 (-28,71, 23.36) | 4.86 (1.17, 20.20) |
| Lung clearance index | 0.07 (-0.38, 0.52) | 4.00 (0.43, 36.73) |

# Variables log transformed

## Supplementary information

### Table S1 Confounders and interactions included in the models for each lung function outcome

| Lung function outcome at 1 year | Confounders | Interactions |
|---|---|---|
| **Respiratory rate** n.min$^{-1}$<br><br>(log transformed) | respiratory rate at 6 weeks, Z-score weight for age at 1 year*, age at 1 year (corrected for gestation), height at 1 year, ethnicity and SES at 1 year | age at 1 year and height at 1 year, age at 1 year squared (corrected for gestation) |
| **Tidal volume** mL (raw score) | tidal volume at 6 weeks, Z-score weight for age at 1 year*, gender, ethnicity and SES at 1 year | age at 1 year to the power of 3 (corrected for gestation) |
| **$t_{PTEF}/t_E$ %** (log transformed) | (tPTEF/tE) 6 weeks, Z-score weight for age at 1 year (according to WHO reference equations), birth weight and gender | age at 1 year squared (corrected for gestation) |
| **Functional residual capacity** mL (raw score) | FRC at 6 weeks, Z-score weight for age at 1 year*, Z score length for age at 1 year*, birth weight, birth length, gender, age at 1 year and maternal alcohol | age at 1 year squared (corrected for gestation), interactions between FRC at 6 weeks, Z-score weight for age at 1 year, Z score length for age at 1 year and gender |
| **Lung clearance index** (raw score) | Z-score weight for age at 1 year*, age at 1 year (corrected for gestation), ethnicity, gender and SES at 1 year | None |
| **Exhaled nitric oxide** (raw score) | Exhaled nitric oxide at 6 weeks, Z-score weight for age at 1 year* | age at 1 year to the power of 3 (corrected for gestational age) |

* Calculated using the WHO Child Growth Standards “I grow up” STATA package.[20]

## Table S2 Determinants of respiratory rate (breaths per minute) at 1 year

| | % Change in respiratory rate | 95% Confidence interval | p-value | R-sq (adjusted) |
|---|---|---|---|---|
| **LRTI[1] (Y/N)** | **1.06** | **(1.01, 1.10)** | **0.010** | **0.15** |
| **Number of LRTI episodes (0-4)** | **1.05** | **(1.02, 1.08)** | **0.001** | **0.17** |
| **Hospitalized LRTI (Y/N)** | **1.10** | **(1.03, 1.17)** | **0.007** | **0.16** |
| Severe LRTI | 1.09 | (0.99, 1.19) | 0.088 | 0.14 |
| RSV (Y/N) | 1.02 | (0.96, 1.07) | 0.588 | 0.14 |
| Household smoke exposure | 1.00 | (0.96, 1.04) | 0.891 | 0.13 |
| Maternal smoking | 0.99 | (0.95, 1.04) | 0.910 | 0.13 |
| **Weight for age Z- score (WAZ)** | 0.98 | (0.96, 0.99) | 0.100 | 0.15 |
| Change in weight for age Z-score[3] | 1.00 | (0.98, 1.02) | 0.696 | 0.15 |
| **Height for age Z- score** | 1.00 | (0.99, 1.01) | 0.913 | 0.13 |
| Change in height for age Z-score[3] | 1.01 | (0.99, 1.02) | 0.740 | 0.15 |
| **Upper quartile of respiratory rate at 6 weeks[4]** | **1.08** | **(1.04, 1.12)** | **<0.001** | **0.08** |
| Preterm (gestation <37 weeks) | 0.94 | (0.88, 1.00) | 0.060 | 0.17 |
| Very preterm (gestation < 32 weeks) | 1.10 | (0.91, 1.30) | 0.306 | 0.16 |
| **Maternal HIV positive** | **1.06** | **(1.01, 1.11)** | **0.028** | **0.17** |
| Maternal alcohol use | 0.99 | (0.89, 1.09) | 0.810 | 0.15 |
| Male sex | 0.99 | (0.96, 1.03) | 0.886 | 0.15 |

[1]Lower respiratory tract infection, [2] Respiratory syncytial virus, [3]Change in z-score from 6 week and 1 year, [4]Derived from normative equations at 6 weeks, upper quartile > 50.6 (breaths per minute)

**Table S3 Determinants of tidal volume ($V_T$) at 1 year**

| | Mean change in $V_T$ mL | 95% Confidence interval | p-value | R-sq (adjusted) |
|---|---|---|---|---|
| **LRTI[1] (Y/N)** | **-3.57** | **(-6.60, -0.54)** | **0.021** | **0.41** |
| **Number of LRTI episodes (0-4)** | **-2.49** | **(-4.65, -0.33)** | **0.024** | **0.41** |
| **Hospitalized LRTI (Y/N)** | **-5.94** | **(-11.23, -0.65)** | **0.028** | **0.41** |
| Severe LRTI | -8.24 | (-17.51, 1.03) | 0.081 | 0.41 |
| RSV[2] (Y/N) | -1.73 | (-5.69, 2.23) | 0.390 | 0.40 |
| Household smoke exposure | 0.49 | (-2.50, 3.48) | 0.747 | 0.40 |
| Maternal smoking | 0.85 | (-2.29, 3.98) | 0.594 | 0.40 |
| **Weight for age Z- score** | **4.16** | **(3.02, 5.30)** | **<0.001** | **0.40** |
| **Change in weight for age Z-score[3]** | **3.26** | **(1.97, 4.56)** | **<0.001** | **0.34** |
| **Height for age Z- score** | **2.83** | **(1.60, 4.06)** | **<0.001** | **0.33** |
| Change in height for age Z-score[3] | **1.22** | **(0.14, 2.29)** | **0.027** | **0.29** |
| **Lower quartile of $V_T$ at 6 weeks[4]** | **-8.64** | **(-12.29, -4.97)** | **<0.001** | **0.13** |
| Preterm (gestation <37 weeks) | -0.22 | (-4..83, 4.39) | 0.924 | 0.28 |
| Very preterm (gestation < 32 weeks) | -5.80 | (-20.41, 8.82) | 0.436 | 0.28 |
| Maternal HIV | 0.70 | (-3.41, 4.82) | 0.737 | 0.28 |
| Maternal alcohol use | -1.04 | (-8.40, 6.33) | 0.782 | 0.28 |
| Male sex | **3.20** | **(0.64, 5.75)** | **0.014** | **0.40** |

[1]Lower respiratory tract infection, [2] Respiratory syncytial virus, [3]Change in z-score from 6 week and 1 year, [4]Derived from normative equations at 6 weeks, lower quartile < 32.2 mL

**Table S4 Determinants of time to peak tidal expiratory flow over total expiratory time ($t_{PTEF}/t_E$) at 1 year**

| | % change in $t_{PTEF}/t_E$ | 95% Confidence interval | p-value | R-sq (adjusted) |
|---|---|---|---|---|
| LRTI[1] (Y/N) | 1.07 | (0.98, 1.16) | 0.148 | 0.22 |
| Number of LRTI episodes (0-4) | 1.03 | (0.97, 1.10) | 0.333 | 0.22 |
| Hospitalized LRTI (Y/N) | 1.01 | (0.85, 1.16) | 0.929 | 0.21 |
| RSV[2] (Y/N) | 1.02 | (0.92, 1.12) | 0.647 | 0.22 |
| Household smoke exposure | 0.99 | (0.91, 1.08) | 0.933 | 0.22 |
| Maternal smoking | 0.99 | (0.92, 1.08) | 0.829 | 0.22 |
| Weight for age Z-score (WAZ) | 0.97 | (0.93, 1.00) | 0.078 | 0.22 |
| **Change in weight for age Z-score[3]** | **0.96** | **(0.92, 1.00)** | **0.026** | **0.22** |
| Height for age Z- score (HAZ) | 0.97 | (0.93, 1.00) | 0.093 | 0.22 |
| Change in height for age Z-score[3] | **0.97** | **(0.94, 1.00)** | **0.055** | **0.22** |
| **Lower quartile of $t_{PTEF}/t_E$ at 6 wks[4]** | **0.76** | **(0.55, 0.77)** | **<0.001** | **0.14** |
| Preterm (gestation <37 weeks) | 0.98 | (0.84, 1.11) | 0.753 | 0.21 |
| Very preterm (gestation < 32 weeks) | 0.91 | (0.51, 0.70) | 0.650 | 0.21 |
| Maternal HIV positive | 1.05 | (0.95, 1.15) | 0.293 | 0.20 |
| Maternal alcohol use | 1.02 | (0.81, 1.22) | 0.886 | 0.19 |
| Male sex | 1.02 | (0.96, 1.08) | 0.547 | 0.19 |

[1]Lower respiratory tract infection, [2] Respiratory syncytial virus, [3]Change in z-score from 6 week and 1 year, [4]Lower quartile < 26.8%

**Table S5 Determinants of functional residual capacity (FRC) at 1 year**

| | Mean change in FRC mL | 95% Confidence interval | p-value | R-sq (adjusted) |
|---|---|---|---|---|
| LRTI[1] (Y/N) | -9.05 | (-21.29, 3.20) | 0.147 | 0.38 |
| Number of LRTI episodes (0-4) | -6.21 | (-14.28, 2.36) | 0.155 | 0.37 |
| Hospitalized LRTI (Y/N) | -7.12 | (-27.82, 13.57) | 0.498 | 0.37 |
| RSV[2] (Y/N) | -7.31 | (-25.34, 10.73) | 0.426 | 0.37 |
| Household smoke exposure | 11.00 | (-0.26, 22.27) | 0.056 | 0.37 |
| Mother smoking | -1.80 | (-13.39, 9.78) | 0.759 | 0.37 |
| **Weight for age Z- score** | **13.27** | **(6.77, 19.78)** | **<0.001** | **0.33** |
| **Change in weight for age Z-score[3]** | **11.67** | **(6.32, 17.01)** | **<0.001** | **0.36** |
| **Height for age Z-score** | **6.62** | **(0.11, 13.13)** | **0.047** | **0.32** |
| Change in height for age Z-score[3] | 2.19 | (-2.84, 7.19) | 0.393 | 0.36 |
| **Lower quartile of FRC at 6 weeks[4]** | **-12.81** | **(-25.58, -0.05)** | **0.047** | **0.35** |
| Preterm (gestation <37 week) | -12.70 | (-32.20, 6.81) | 0.201 | 0.37 |
| Very preterm (gestation < 32 week) | -35.84 | (-135.78, 72.09) | 0.546 | 0.37 |
| Maternal HIV | 0.29 | (-12.10, 12.67) | 0.964 | 0.38 |
| Maternal alcohol use | -2.68 | (-28.71, 23.36) | 0.886 | 0.37 |
| **Male sex** | **18.94** | **(5.98, 31.89)** | **0.004** | **0.36** |

[1]Lower respiratory tract infection, [2] Respiratory syncytial virus, [3]Change in z-score from 6 week and 1 year, [4]Lower quartile < 64.8 mL

## Table S6 Determinants of lung clearance index (LCI) at 1 year

| | Mean change in LCI (n turnovers) | 95% Confidence interval | p-value | R-sq (adjusted) |
|---|---|---|---|---|
| LRTI[1] (Y/N) | 0.13 | (-0.06, 0.33) | 0.181 | 0.04 |
| **Number of LRTI episodes (1-4)** | **0.13** | **(0.00, 0.26)** | **0.046** | **0.05** |
| Hospitalised LRTI (Y/N) | 0.28 | (-0.04, 0.59) | 0.088 | 0.04 |
| RSV[2] (Y/N) | 0.04 | (-0.08, 0.17) | 0.494 | 0.03 |
| **Household smoke** | **0.19** | **(0.00, 0.39)** | **0.046** | **0.05** |
| **Mother smoking** | 0.06 | (-0.15, 0.26) | 0.598 | 0.03 |
| **Weight for age Z-score (WAZ)[3]** | **-0.13** | **(-0.21, -0.06)** | **<0.001** | **0.04** |
| **Change in weight for age Z-score[3]** | **-0.09** | **(-0.17, 0.00)** | **0.043** | **0.01** |
| Height for age Z- score (HAZ)[3] | 0.02 | (-0.07, 0.11) | 0.685 | 0.03 |
| Change in height for age Z-score[3] | -0.02 | (-0.10, 0.05) | 0.529 | 0.01 |
| Increased LCI at 6 weeks[4] | 0.05 | (-0.14, 0.25) | 0.592 | 0.04 |
| Preterm (gestation <37 weeks) | 0.04 | (-0.22, 0.31) | 0.743 | 0.03 |
| Very preterm (gestation < 32 weeks) | 0.21 | (-0.52, 0.96) | 0.569 | 0.03 |
| Maternal HIV | 0.18 | (-0.06, 0.41) | 0.135 | 0.04 |
| Maternal alcohol use | 0.07 | (-0.38, 0.52) | 0.753 | 0.03 |
| Male sex | 0.03 | (-0.05, 0.10) | 0.483 | 0.03 |

[1]Lower respiratory tract infection, [2] Respiratory syntial virus, [3]Change in z-score from 6 week and 1 year, [4]Upper quartile LCI > 7.4

**Table S7 Determinants of exhaled nitric oxide (eNO) at 1 year**

| | Mean change in eNO (ppm) | 95% Confidence interval | p-value | R-sq (adjusted) |
|---|---|---|---|---|
| LRTI[1] (Y/N)) | -0.69 | (-3.44, 2.06) | 0.620 | 0.07 |
| Number of LRTI episodes (1-4) | -1.01 | (-3.00, 0.96) | 0.312 | 0.07 |
| Hospitalised LRTI (Y/N) | -1.01 | (-5.81, 3.79) | 0.680 | 0.07 |
| RSV[2] (Y/N) | -1.34 | (-4.86, 2.18) | 0.454 | 0.07 |
| Household smoke exposure | 1.17 | (-1.35, 3.70) | 0.361 | 0.07 |
| Mother smoking | 0.47 | (-2.03, 2.96) | 0.712 | 0.07 |
| **Weight for age Z-score (WAZ)[3]** | **-0.92** | **(-1.83, -0.02)** | **0.046** | **0.07** |
| **Change in weight for age Z-score[3]** | **-1.10** | **(-2.17, -0.02)** | **0.045** | **0.07** |
| Height for age Z- score (HAZ)[3] | -0.78 | (-1.76, 0.21) | 0.121 | 0.06 |
| Change in height for age Z-score[3] | -0.67 | (-1.53, 0.19) | 0.129 | 0.06 |
| **Increased eNO at 6 weeks[4]** | **4.43** | **(1.58, 7.29)** | **0.002** | **0.05** |
| Preterm (gestation <37 weeks) | 1.98 | (-1.63, 5.60) | 0.280 | 0.07 |
| Very preterm (gestation < 32 weeks) | 5.95 | (-3.50, 15.40) | 0.216 | 0.07 |
| Maternal HIV | 0.53 | (-2.45, 3.52) | 0.725 | 0.07 |
| Maternal alcohol use | 2.38 | (-3.57, 8.32) | 0.432 | 0.07 |
| Male sex | -0.35 | (-2.68, 1.99) | 0.770 | 0.07 |

[1]Lower respiratory tract infection, [2] Respiratory syntial virus, [3]Change in z-score from 6 week and 1 year, [4]Upper quartile eNO 11.61 ppb

**Table S8: Predictors of respiratory rate (breaths per minute): middle quartiles compared to upper (>29.7) and lower (27.4) quartiles**

| | Upper quartile | Middle quartiles | Lower quartile | OR (95% CI) p-value lower: 2 middle quartiles | OR (95% CI) p-value upper: 2 middle quartiles |
|---|---|---|---|---|---|
| **LRTI[1] (Y/N)** | **31.82% (28/88)** | **17.73% (25/141)** | 25.33% (19/75) | 1.57 (0.80, 3.10) p=0.189 | **2.17(1.16, 4.04) p=0.015** |
| **Number LRTI episodes (0-4)** | **0.32 (0.47)** | **0.18 (0.38)** | 0.25 (0.44) | 1.25 (0.81, 1.91) p=0.314 | **1.50 (1.01, 2.25) p=0.046** |
| Hospitalized LRTI1 (Y/N) | 7.95% (7/88) | 5.67% (8/141) | 12.00% (9/75) | 2.27 (0.84, 6.14) p=0.108 | 1.44 (0.50, 4.11) p=0.499 |
| Number hospitalized episodes (0-3) | 0.10 (0.40) | 0.06 (0.23) | 0.16 (0.49) | 2.32 (0.98, 5.47) p=0.055 | 1.60 (0.67, 3.83) p=0.292 |
| **RSV (Y/N)** | 13.64% (12/88) | **7.80% (11/141)** | **17.33% (13/75)** | **2.48 (1.05, 5.84) p=0.038** | 1.87 (0.79, 4.43) p=0.158 |
| Household smoking | 60.92% (53/87) | 64.54% (91/141) | 76.00% (57/75) | 1.74 (0.92, 3.27) p=0.086 | 0.86 (0.49, 1.49) p=0.582 |
| Maternal smoking | 25.29% (22/87) | 34.75% (49/141) | 36.00% (27/75) | 1.06 (0.59, 1.90) p=0.855 | 0.64 (0.35, 1.15) p=0.135 |
| Change in weight for age Z-score | 0.45 (1.04) | 0.25 (1.14) | 0.34 (1.09) | 0.93 (0.70, 1.22) p=0.587 | 1.06 (0.82, 1.36) p=0.683 |
| Change in height for age Z-score[2] | 0.48 (1.35) | 0.31 (1.39) | -0.03 (1.22) | 0.82 (0.65, 1.05) p=0.112 | 1.10 (0.90, 1.34) p=0.368 |
| **Upper quartile of respiratory rate at 6 weeks** | **42.05% (37/88)** | **25.53% (36/141)** | **8.00% (6/75)** | **0.25 (0.10, 0.63) p=0.003** | **2.12 (1.20, 3.73) p=0.010** |
| Preterm (<37 weeks) | 19.32% (17/88) | 10.64% (15/141) | 17.33% (13/75) | 1.76 (0.79, 3.93) p=0.167 | 2.01 (0.95, 4.27) p=0.069 |
| Very preterm (< 32 weeks) | 3.41% (3/88) | 0.77% (1/141) | 2.67% (2/75) | 3.84 (0.34, 43.01) p=0.276 | 4.94 (0.51, 48.27) p=0.169 |
| Maternal HIV | 27.27% (24/88) | 18.44% (26/141) | 14.67% (11/75) | 0.76 (0.35, 1.64) p=0.484 | 1.66 (0.88, 3,13) p=0.117 |
| Maternal alcohol use | 2.47% (2/81) | 3.82% (5/131) | 7.35% (5/68) | 2 (0.55, 7.16) P=0.287 | 0.64 (0.12, 3.37) p=0.596 |

[1]Lower respiratory tract infection

**Table S9: Predictors of tidal volume in the lower quartile at 1 year (<89.68 mL)**

| | Lower quartile | Upper quartile | Odds ratio | 95% Confidence interval | p-value |
|---|---|---|---|---|---|
| LRTI[1] (Y/N) | 28.95% (22/76) | 25.00% (22/76) | 1.22 | (0.60, 2.51) | p=0.584 |
| Number of LRTI episodes (0-4) | 0.39 (0.71) | 0.32 (0.68) | 1.18 | (0.74, 1.88) | p=0.484 |
| Hospitalized LRTI (Y/N) | 11.84% (9/76) | 7.89% (6/76) | 1.57 | (0.53, 4.64) | p=0.417 |
| Number hospitalized episodes (0-3) | 0.16 (0.49) | 0.08 (0.27) | 1.72 | (0.70, 4.25) | p=0.236 |
| RSV (Y/N) | 10.53% (8/76) | 18.42%(14/76) | 1.92 | (0.75, 4.89) | p=0.171 |
| Household smoking | 72.37% (55/76) | 57.89%(44/76) | 1.90 | (0.97, 3.75) | p=0.063 |
| Maternal smoking | 43.42% (33/76) | 28.95% (22/76) | 1.88 | (0.96, 3.69) | p=0.065 |
| **Change in weight for age Z-score** | **-0.57 (0.93)** | **1.08 (0.80)** | **0.09** | **(0.04, 0.20)** | **p<0.001** |
| **Change in height Z score between 1 year and 6 weeks** | **-0.15 (1.44)** | **0.63 (1.38)** | **0.66** | **(0.50, 0.87)** | **p=0.003** |
| **Lower quartile of tidal volume at 6 weeks** | **39.13% (27/69)** | **10.77% (7/65)** | **5.33** | **(2.12, 13.38)** | **p<0.001** |
| **Preterm (<37 weeks)** | **30.26% (23/76)** | **14.47% (11/76)** | **2.56** | **(1.15, 5.73)** | **p=0.022** |
| **Very preterm (< 32 weeks)** | **3.95% (3/76)** | **1.32% (1/76)** | **3.08** | **(0.31, 30.31)** | **p=0.334** |
| **Maternal HIV** | **13.16% (10/76)** | **26.32% (20/76)** | **0.42** | **(0.18, 0.98)** | **p=0.045** |
| Maternal alcohol use | 10.00% (7/70) | 1.45% (1/69) | 7.55 | (0.90, 63.14) | p=0.062 |

[1]Lower respiratory tract infection

**Table S10: Predictors of $t_{PTEF}/t_E$ in the lowest quartile at 1 year (< 25.28 %) and (>31.50%) the highest quartile, results presented as % (n) or mean (s.d.)**

| | Upper quartile | 2 Middle quartiles | Lower quartile | OR (95% CI) p-value lower: middle quartiles | OR (95% CI) p-value upper: middle quartiles |
|---|---|---|---|---|---|
| LRTI[1] (Y/N) | 24.00% (24/100) | 21.01% (29/138) | 28.79% (19/66) | 1.52 (0.76, 2.98) p=0.222 | 1.19 (0.64, 2.20) p=0.585 |
| Number of LRTI episodes (0-4) | 0.38 (0.83) | 0.38 (0.72) | 0.25 (0.51) | 1.44 (0.89, 2.34) p=0.139 | 1.35 (0.91, 2.01) p=0.131 |
| Hospitalized LRTI (Y/N) | 10.00% (10/100) | 5.09% (7/138) | 10.61% (7/66) | 2.22 (0.75, 6.62) p=0.152 | 2.08 (0.76, 5.67) p=0.152 |
| Number of hospitalized episodes (0-3) | 0.14 (0.49) | 0.12 (0.37) | 0.05 (0.22) | 2.30 (0.84, 6.31) p=0.105 | 2.10 (0.91, 4.87) p=0.084 |
| RSV (Y/N) | 15.00% (15/100) | 7.97% (11/138) | 15.15% (10/66) | 2.06 (0.83, 5.13) p=0.120 | 2.04 (0.89, 4.4.64) p=0.091 |
| Household smoking | 60.61% (60/99) | 66.67% (92/138) | 74.24% (49/66) | 1.44 (0.75, 2.76) p=0.274 | 0.77 (0.45, 1.32) p=0.338 |
| Maternal smoking | 25.25% (25/99) | 34.78% (48/138) | 37.88% (25/66) | 1.14 (0.62, 2.10) p=0.666 | 0.63 (0.36, 1.12) p=0.118 |
| **Difference in weight for age Z- score** | **0.10 (1.05)** | **0.45 (1.18)** | 0.41 (1.04) | 1.03 (0.79, 1.35) P=0.821 | **0.75 (0.57, 0.99) p=0.044** |
| Difference in height for age Z- score | 0.05 (1.34) | 0.53 (1.49) | 0.13 (1.28) | 1.14 (0.91, 1.41) p=0.252 | 0.86 (0.69, 1.08) p=0.190 |
| Preterm (<37 weeks) | 14.00% (14/100) | 14.49% (20/138) | 16.67% (11/66) | 1.18 (0.53, 2.63) p=0.686 | 0.96 (0.46, 2.01) p=0.915 |
| Very preterm (< 32 weeks) | 3.00% (3/100) | 1.45% (2/138) | 1.52% (1/66) | 1.05 (0.09, 11.75) p=0.971 | 2.10 (0.34, 12.83) p=0.420 |
| **Maternal HIV** | **29.00% (29/100)** | **17.39% (24/138)** | 12.12% (8/66) | 0.66 (0.28, 1.55) p=0.335 | **1.94 (1.05, 3.59) p=0.035** |
| Maternal alcohol use | 3.17% (4/126) | 2.17% (2/92) | 9.68% (6/62) | 3.27 (0.89, 12.04) p=0.075 | 0.68 (0.12, 3.78) p=0.657 |

[1]Lower respiratory tract infection

**Table S11: Predictors of lower quartile functional residual capacity at 1 year (< 177.18 mL), results presented as % (n) or mean (s.d.)**

| | Upper quartile | 2 Middle quartiles | Lower quartile | OR (95% CI) p-value lower: middle quartiles | OR (95% CI) p-value upper: middle quartiles |
|---|---|---|---|---|---|
| LRTI[1] (Y/N) | 24.11% (27/112) | 25.00% (32/128) | 20.31% (13/64) | 0.76 (0.37, 1.58) p=0.471 | 0.95 (0.53, 1.72) p=0.873 |
| Number of LRTI episodes (0-4) | 0.38 (0.83) | 0.31 (0.60) | 0.23 (0.50) | 0.77 (0.44, 1.36) p=0.369 | 1.13 (0.79, 1.62) p=0.500 |
| Hospitalized LRTI (Y/N) | 9.82% (11/112) | 7.81% (10/128) | 4.69% (3/64) | 0.58 (0.15, 2.19) p=0.421 | 1.29 (0.52, 3.15) p=0.583 |
| Number hospitalized episodes (0-3) | 0.10 (0.30) | 0.08 (0.27) | 0.05 (0.21) | 0.56 (0.16, 1.97) p=0.369 | 1.37 (0.70, 2.67) p=0.354 |
| RSV (Y/N) | 13.39% (15/112) | 10.94% (14/128) | 10.94% (7/64) | 1 (0.38, 2.61) p=1.00 | 1.26 (0.58, 2.74) p=0.561 |
| Household smoking | 63.06% (70/111) | 64.06% (82/128) | 76.56% (49/64) | 1.83 (0.93, 3.63) p=0.082 | 0.96 (0.56, 1.62) p=0.873 |
| **Maternal smoking** | 30.63% (34/111) | **28.13% (36/128)** | **43.75% (28/64)** | **1.99 (1.06, 3.72) p=0.032** | 1.13 (0.65, 1.97) p=0.671 |
| **Difference in weight for age Z- score** | **0.81 (1.03)** | **0.38 (1.00)** | **-0.36 (0.95)** | **0.47 (0.34, 0.66) p<0.001** | **1.53 (1.15, 2.04) p=0.004** |
| **Difference in height for age Z- score** | 0.62 (1.22) | 0.62 (1.22) | -0.10 (1.37) | **1.23 (1.00, 1.53) p=0.053** | 1.10 (0.90, 1.34) p=0.368 |
| **Lower quartile of FRC at 6 weeks** | 20.24% (17/84) | **21.88% (28/128)** | **40.63% (26/64)** | **0.83 (0.76, 0.91) p<0.001** | 1.06 (0.98, 1.14) p=0.162 |
| Preterm (<37 weeks) | 16.07% (18/112) | 13.28% (17/128) | 15.63% (10/64) | 1.21 (0.52, 2.82) p=0.660 | 1.25 (0.61, 2.56) p=0.542 |
| Very preterm (< 32 weeks) | 2.68% (3/112) | 0.78% (1/128) | 3.13% (2/64) | 4.10 (0.36, 46.05) p=0.253 | 3.50 (0.36, 34.09) p=0.282 |
| **Maternal HIV** | 25.00% (28/112) | **21.88% (28/128)** | **7.81% (5/64)** | **0.30 (0.11, 0.83) p=0.020** | 1.19 (0.65, 2.16) p=0.568 |
| **Maternal alcohol use** | 2.91% (3/103) | **2.46% (3/122)** | **10.91% (6/55)** | **4.86 (1.17, 20.20) p=0.030** | 1.19 (0.23, 6.03) p=0.834 |

[1]Lower respiratory tract infection

**Table S12: Predictors of upper quartile lung clearance index (LCI) at 1 year (LCI > 6.88), results presented as % (n) or mean (s.d.)**

| | Upper quartile | Lower quartile | Odds ratio | 95% Confidence interval | p-value |
|---|---|---|---|---|---|
| LRTI[1] (Y/N) | 24.66% (18/73) | 25.00% (19/76) | 0.98 | (0.47, 2.07) | p=0.961 |
| Number of LRTI episodes (0-4) | 0.32 (0.64) | 0.32 (0.66) | 1.00 | (0.61, 1.64) | p=0.995 |
| Hospitalized LRTI (Y/N) | 8.22% (6/73) | 7.89% (6/76) | 1.04 | (0.32, 3.40) | p=0.942 |
| Number hospitalized episodes (0-3) | 0.12 (0.47) | 0.08 (0.27) | 1.37 | (0.57, 3.32) | p=0.483 |
| **RSV (Y/N)** | **19.18% (14/73)** | **7.89% (6/76)** | **2.77** | **(2.00, 7.66)** | **p=0.050** |
| Household smoking | 71.23% (52/73) | 69.74% (53/76) | 1.07 | (0.53, 2.17) | p=0.841 |
| Maternal smoking | 36.99% (27/73) | 38.16% (29/76) | 0.95 | (0.49, 1.85) | p=0.883 |
| **Difference in weight for age Z- score** | **-0.64 (0.94)** | **1.21 (0.82)** | **0.08** | **(0.04, 0.19)** | **p<0.001** |
| **Difference in height for age Z- score** | **-0.38 (1.41)** | **0.87 (1.40)** | **0.50** | **(0.37, 0.69)** | **p<0.001** |
| Lower quartile of tidal volume at 6 weeks | 22.58% (14/62) | 26.56% (17/64) | 0.81 | (0.36, 1.82) | p=0.604 |
| Preterm (<37 weeks) | 17.81% (13/73) | 19.74% (15/76) | 0.88 | (0.39, 2.01) | p=0.763 |
| Very preterm (< 32 weeks) | 2.74% (2/73) | 1.32% (1/76) | 2.11 | (0.19, 23.81) | p=0.545 |
| Maternal HIV | 17.81% (13/73) | 21.05% (16/76) | 0.81 | (0.36, 1.83) | p=0.617 |
| Maternal alcohol use | 5.63% (4/71) | 1.47% (1/68) | 4.00 | (0.43, 36.73) | p=0.220 |

[1]Lower respiratory tract infection

**Table S13: Predictors of upper quartile exhaled nitric oxide (eNO) at 1 year, results presented as % (n) or mean (s.d.)**

| | Upper quartile | Lower quartile | Odds ratio | 95% Confidence interval | p-value |
|---|---|---|---|---|---|
| LRTI[1] (Y/N) | 14.93% (10/67) | 23.88% (16/67) | 0.56 | (0.23, 1.34) | 0.193 |
| Number of LRTI episodes (0-4) | 0.19 (0.53) | 0.28 (0.55) | 0.73 | (0.38, 1.40) | 0.338 |
| Hospitalized LRTI (Y/N) | 4.48% (3/67) | 7.46% (5/67) | 0.58 | (0.13, 2.54) | 0.470 |
| Number hospitalized episodes (0-3) | 0.06 (0.30) | 0.07 (0.36) | 0.82 | (0.24, 2.81) | 0.757 |
| RSV (Y/N) | 8.96% (6/67) | 8.96% (6/67) | 1.00 | (0.32, 3.27) | 1.000 |
| Household smoking | 71.64% (48/67) | 67.16% (45/67) | 1.24 | (0.59, 2.58) | 0.574 |
| Maternal smoking | 40.30% (27/67) | 32.84% (22/67) | 1.38 | (0.68, 2.80) | 0.370 |
| **Difference in weight for age Z- score** | **-0.01 (1.40)** | **0.69 (1.30)** | **0.46** | **(0.32, 0.66)** | **<0.001** |
| **Difference in height for age Z- score** | **-0.15 (1.13)** | **0.77 (1.06)** | **0.67** | **(0.51, 0.89)** | **0.005** |
| **Upper quartile eNO at 6 weeks** | **48.48% (32/66)** | **2.99% (2/67)** | **30.59** | **(6.91, 135.39)** | **<0.001** |
| Preterm (<37 weeks) | 17.91% (12/67) | 13.43% (9/67) | 0.71 | (0.28, 1.82) | 0.477 |
| Very preterm (< 32 weeks) | 5.97% (4/67) | 0.00% (0/67) | N/A | N/A | N/A |
| Maternal HIV | 17.91% (12/67) | 25.37% (17/67) | 0.64 | (0.28, 1.48) | 0.296 |
| Maternal alcohol use | 1.64% (1/61) | 4.76% (3/63) | 3.00 | (0.30, 29.66) | 0.347 |

[1]Lower respiratory tract infection

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Chapter 7

# Summary and recommendations

This work investigated lung function and growth in African infants in the context of a birth cohort study and the antenatal and early life factors impacting this. Comprehensive measures of lung function were established and performed at six weeks and at one year of age. Several important risk factors for altered lung function were identified many of which are amenable to public health intervention.

We obtained the first infant lung function data in African children, showing the feasibility of establishing this testing in a LMIC setting and the utility of this. Importantly, the testing success rate was high with success in unsedated six-week infants comparable to that published in European cohorts, [1, 2] and with unprecedented success rates in unsedated one year olds. Key reasons for this include collaboration with experts, well trained, skilled staff, quality control procedures, a dedicated testing space; resources for equipment and staffing and strong community engagement.

We used a comprehensive set of lung function measures, selecting tests which could be done safely during tidal breathing in unsedated infants: tidal breathing measures of volume, flow and respiratory rate; exhaled nitric oxide as a measure of airway inflammation and inert gas MBW testing to measure lung growth and ventilation homogeneity. These tests were useful in detecting the impact of antenatal and early life exposures on lung function both at six weeks and at one year in the context of a birth cohort. We were able to successfully test a large number of unsedated infants at one year allowing us to measure lung function longitudinally without having to account for the effect of sedation on lung function. We also used the FOT, measuring respiratory system resistance and compliance. The FOT has only very recently been developed for use during tidal breathing in unsedated infants.[3] This research validated the use of this test in unsedated six week infants and as a measure sensitive enough to detect the effects of exposures such as maternal smoking in pregnancy.[4]

The utility of any lung function measure to distinguish between health and disease in a population or to detect a significant alteration in measured outcome requires a robust normative standard, including knowledge of individual and population level variability. Such standards are lacking in lung function outcomes in African infants, children and

adults.[5] This research has contributed to the development of normative data for healthy African infants.[6] A limitation of this normative data is the narrow age range in which lung function was measured. This limits generalizability of these reference data to older children, as lung function is strongly associated with age and growth in early life.[7] In addition, these data are relevant to children from similar populations with similar exposures, and may not be generalizable to other African populations from different socioeconomic settings and with differing environmental exposures. However this reference data may be useful in epidemiological studies in similar populations and in longitudinally assessing the impact of early life exposures on lung health. Given that children from LMICs are at particular risk of respiratory disease,[8] robust epidemiological research that improves our understanding of the impact of early exposures on lung growth and function in these settings is key.

**Factors associated with lung function at six weeks**

The study identified a number of antenatal and early life exposures that were associated with lung function at six weeks of age, some factors that have been previously described and some novel. The impact of infant sex, birth weight, ethnicity and maternal smoking in this study were consistent with previous reports.[9-13] Male infants had lower tidal expiratory flow ratios and respiratory system compliance despite having larger lung volumes compared to female infants, suggesting that male infants have relatively smaller airways to lung size. These differences between male and female lung function persisted to one year, as has been previously described in non-African infants.[10] Low birth-weight was independently associated with lower respiratory system compliance and smaller FRC in early life, highlighting the importance of the in utero environment on, not only somatic, but also lung growth.

Tobacco smoke exposure, which is known to impair lung growth and function in early life, was very common in our population with 32% of infants having mothers that smoked during pregnancy and 66% of infants exposed to environmental tobacco smoke postnatally. Infants whose mothers smoked during pregnancy had impaired lung function at six weeks, with smaller size adjusted lung volumes, increased lung clearance index and reduced respiratory system compliance compared to infants whose mothers did not

smoke. This suggests that exposure to antenatal and very early life environmental tobacco smoke not only affects lung growth, but may have early damaging effects on small airways with reduced ventilation homogeneity.

There were a number of novel findings in the study such as the impact of indoor air pollutant benzene, maternal alcohol intake during pregnancy and maternal HIV infection on lung function at six weeks. These are especially important findings, as these factors are highly prevalent in Sub-Saharan Africa. One of the strengths of this study is the inclusion of comprehensive measures of the infants' home environment.[14] Benzene, one of the indoor pollutants with high levels in this cohort, was associated with reduced expiratory flow ratios soon after birth. This supports the sparse infant lung function literature that describes altered lung function with exposure to air pollution[15] and highlights the importance of the child's environment in optimizing respiratory health. Maternal health is important for healthy pregnancies.

Maternal factors that were associated with altered lung function in our study were maternal alcohol use in pregnancy and maternal HIV infection. In utero alcohol exposure was found to be a strong independent risk factor for altered lung function at six weeks. Although described in animal studies[16, 17], altered lung function in early life after in utero alcohol exposure has not been previously described in human studies. This interaction requires further study. Infants of mothers with HIV infection had higher size adjusted tidal volumes at six weeks with higher minute ventilation compared to infants whose mothers were HIV uninfected; a difference which persisted to one year. Whether this is related to exposure to the HIV virus itself, antiretroviral therapy or differing infant feeding practices is not known and requires further research.

Some of the mechanisms behind the association of antenatal and early life exposures and impaired early lung function have been identified through epidemiological and physiological studies. For example, intrauterine growth retardation may be caused by impaired trans-placental supply of oxygen and nutrients, which leads to longstanding structural changes of the lungs including decreased surfactant activity and impaired alveolar cell maturation.[18, 19] Reduction in lung fluid and foetal breathing movements also leads

to impaired lung growth. As expected we found an independent effect of low birth weight and lung volumes in this cohort. Smoke exposure antenatally and early life may alter control of breathing.[20] This together with decreased plancental transfer of oxygen across the placenta may be responsible for reduced fetal breathing movements, impairing lung growth.[20] Smoke exposure is also associated with thickened airway walls,[21, 22] which would contribute to decreased airway complaince found in this cohort. Airway number and size may be impaired by early life smoke exposure,[23] which could contribute to the altered lung volumes and impaired ventilation homogeneity seen in this study. *In utero* alcohol exposure impacts the developing fetal brain, providing a possible basis for altered control of breathing in alcohol exposed infants. In addition *in utero* alcohol exposure reduces surfactant protein and alveolar macrophage function and increases lung susceptibility to trauma and infection in animal models.[24, 25] Animal studies have also shown alcohol exposure during pregnancy to impact lung growth and structure[16], although some do not persist into early postnatal life.[26] These mechanisms may explain the altered respiratory rate and expiratory flow ratio and mildly reduced tidal volumes seen in alcohol exposed infants in this cohort, although this effect needs to be confirmed in a larger cohort of alcohol exposed infants. Other exposures such as exposure to maternal HIV infection require further study to improve our understand of possible underlying mechanisms and the relevance of these exposures on longer-term lung function and growth.

**Factors associated with lung function at one year**

Lung function was found to track from six weeks to one year of age, consistent with studies from high-income countries.[10] Factors impacting normal lung growth during the first year of life included somatic growth, exposure to environmental tobacco smoke, maternal HIV infection, maternal alcohol intake during pregnancy and early life lower respiratory tract infections (LRTI).

Increased postnatal weight gain was associated with reduced expiratory flows at one year, as has been previously described.[27] Environmental tobacco smoke exposure during the first year of life was associated with reduced lung volumes at one year independent of the previously discussed effects on six week lung function. Given that low lung function in infancy is associated with respiratory illness in later life[28, 29], this has public health

implications particularly in the context of the rising prevalence of smoking in LMIC such as South Africa.[30] Maternal alcohol remained an independent predictor of low lung volumes at one year and the increased minute ventilation noted in HIV exposed infants at six weeks persisted to one year of age even after adjusting for growth and confounding factors.

Early life LRTI were identified as a key factor in reducing lung function at one year, independent of baseline lung function. This is true for even ambulatory non-severe respiratory tract infections. The effect was stronger for more severe LRTI, requiring hospitalisation and each additional LRTI led to further reduction in one-year lung function outcomes. This is a novel finding as the sparse longitudinal infant lung function data available, suggests that low lung function after early life respiratory infections is mediated by prior low lung function. However, these studies were completed in very different populations with a lower rate of respiratory tract infections, higher socioeconomic status and likely differing epidemiology of respiratory infections. Given the burden of LRTI in African children, this finding is particularly important.

**Future research should address the following areas:**

1. Longitudinal follow-up of this cohort to further investigate the normal lung growth and development through infancy, preschool and childhood in this African cohort. This will provide valuable reference data against which the impact of exposures and potential interventions can be measured.

2. Longitudinal follow-up of this cohort through childhood to investigate the impact of antenatal and early life factors including LRTI on long-term lung health is important.

3. The pathophysiological mechanisms of exposures and reduced lung function in African infants need to be investigated. For example LRTI may cause direct damage to lung parenchyma; persistent airway inflammation or possible increased bronchial hyper-reactivity. Well designed studies to test such hypotheses, systematic development of tools able to test the relevant aspects of respiratory function and longitudinal normative data such as is being collected in this cohort to which effect size can be related are needed.

4. Development of lung function tests able to be collected in unsedated preschool children and assessment of their clinical utility, including normal variability and defining bronchodilator response in the African setting. The FOT is a promising technique as it has the potential to provide information regarding airway obstruction and bronchodilator response even in small children who are unable to perform respiratory manoeuvres. This work has laid a strong foundation for development of this technique as a longitudinal assessment tool of lung function in infants and preschool children and the potential future development of this technique as a clinical tool in the assessment of respiratory disease in young African children.

We are able to undertake many of these research priorities given the lung function testing capacity that has been developed and the well characterised birth cohort that has been established through the Drakenstein Child Health study who we continue to follow.

**Recommendations**

This study has identified several areas to optimise lung health in African children by maximising early lung growth and function. These include:

5. *Optimising maternal health.* *I*mproving maternal health is important to optimise pregnancy outcomes and maximise child lung health. Maternal HIV infection and maternal alcohol use are two factors the study has identified as determinants of normal lung development. Antenatal programmes aimed at reducing alcohol intake during pregnancy should be considered. Such a programme would need to include good surveillance and targeted individual and group counselling with assessment for referral for specialist services. Strengthening the follow-up of infants exposed to HIV must be priortised.

6. *Reduction in early life respiratory infections* The study has identified LRTI in infancy as an important factor that reduces lung function. Current available preventative interventions such as breast-feeding, optimising nutrition, vaccination against common organisms, prevention of mother to child transmission of HIV infection and reducing exposure to tobacco smoke and environmental pollution should be strength-

ened.[31] In addition, novel interventions to prevent early respiratory infections e.g. vaccination of pregnant women against respiratory syncytial virus [32] are priority areas of research.

7. *Reducing tobacco smoke exposure during pregnancy and in early life* Our data provides further evidence for the importance of smoking prevention as a priority public health intervention. Programmes aimed at smoking cessation in young and pregnant woman should be prioritized. These must include a combination of group therapy, supportive therapy and ensure availability of context specific skilled counselling and appropriate medication.[33] The use of vitamin C during pregnancy may ameliorate the effect of in utero smoke exposure[34], but this should not lessen efforts to reduce smoking in pregnant woman. In addition focussed efforts to reduce smoking in our communities are needed. This is likely to require broad advocacy from public, private and government sectors that includes both smoking prevention and cessation strategies.

8. *Reducing exposure to indoor air pollution* This study found that exposure to indoor air pollution further impacts on infant lung function. Strategies to reduce indoor air pollution, such as improved housing with less overcrowding, adequate ventilation and reliable electricity supply are needed.

**Conclusion**

Infant lung function was feasible, performed with high success rates and a useful epidemiological tool in a South African community setting. Important factors that impact on lung health were identified. These included known exposures as well as novel factors such as HIV exposure or maternal alcohol use. These provide targets for strengthening respiratory health in African children. The use of this objective measure of lung function in African infants has highlighted the importance of population specific data and has laid a foundation for ongoing epidemiological research into the respiratory health of African infants and a base from which to undertake longitudinal follow-up.

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# Appendix A

## Drakenstein Infant Lung Function Study – Consent Form

Thank you for agreeing to be part of the Drakenstein Child Lung Health Study. This form contains more detail about the lung function testing part of the study. This is similar information to that which you may have read in the lung function information leaflet. Although we know you have already signed a consent form at enrolment we want to be sure that you have enough information about the lung function testing and that you are happy for you and your baby to have a lung function test.

### Why is infant lung function being measured?

South African children have a high rate of chest problems such as pneumonia, asthma and cough. We know from studies done in other countries that there are many things that can be a risk for chest problems in young children such as poor growth, exposure to cigarette smoke and other air pollutants and infections. At present we do not know what the most important risks are for South African children nor what is normal lung function for South African babies as it has not been measured before. By measuring a baby's lung function over time we can find out how big their lungs are, how well the lungs work and if they grow normally over time. This study will allow us to find out what is normal lung function in South African babies and will provide important information about what causes chest disease in children and the effect these things have on lung growth and later chronic chest problems. This will help us to understand how best to prevent and treat lung disease in our children.

### What does infant lung function measure?

The test measures your baby's lung size and breathing pattern. There are 4 different tests but two are done at the same time.

### 1. Tidal breathing flow volume test and exhaled nitric oxide test

The first test measures how fast (flow) your baby breaths and how much (volume) they breathe with each normal breath. By doing this the equipment can calculate information

about the growth and working of your babies lungs. At the same time the equipment can measure the amount of a gas called nitric oxide (NO) that is in your baby's breath when they breathe out. NO is a gas that is important in the working of our bodies. We all have NO in the air we breathe out. High levels of NO in our breath can mean our airways or breathing tubes are inflamed. This has been shown to happen with diseases such as asthma, allergies or exposure to pollutants such as cigarette smoke.

**2. Sulphur hexafluoride (SF6) multiple breath washout test**

We can also measure the size of your baby's lungs and the way the different parts of the lungs contribute to breathing. We do this by adding a gas (SF6) that does not get absorbed into the body to the air your baby is breathing. This is breathed for a short period. This gas is not smelly or dangerous to your baby.

**3. Forced oscillation technique**

This test uses sound waves to test how a baby's lungs work and can pick up early problems with a baby's lung growth and function.

**How are the tests done?**

All the tests are done during quiet normal breathing (tidal breathing) when your baby is asleep. Your baby will need to be asleep and, while lying on his/her back, a face mask with air flowing through it will be placed over your baby's nose and mouth. This can take all the measurements mentioned above. Often babies wriggle, sigh or wake and so sometimes we will need to repeat the test. We need to try and get 3 good tests. Depending on how deeply baby is sleeping it can take anywhere from 20 min to 1 hour and may need to be continued after the next feed.

We will repeat these test when your baby is 1year and 2 years old. The tests will be the same and will also be done when your child is sleeping. It is less likely that your child sleep through the tests the older they get. For this reason we will be try and arrange these tests at a time of the day that your child usually sleeps. We will discuss this with you when we arrange the appointment. If your child does not manage to sleep through the testing and if you are happy for us to do so we will give your child some medication

to make him/her sleepy. This medication is called chloral hydrate and makes most children sleepy enough for the testing to be done. This is called sedation. Although chloral hydrate is used commonly to sedate children for various tests and procedures done in hospital there are risks to sedating a child. The most common side effects are: lack of sedation, nausea or prolonged sleepiness. At high doses there are risks of more severe side effects including slowing of breathing, deep sleepiness and an abnormal heart rate. For this reason we will not be using a high dose and we will not repeat the dose of sedation. Sedating your child with chloral hydrate will mean they must wait at the clinic until they have woken up so that we can monitor their heart and breathing rate. This can take up to 2 to 4 hours. We will make sure there is a comfortable place to wait and that you have access to food and drinks should you have to wait. If you do not want your child to be sedated that is absolutely fine. We will attempt testing without sedation as with all children but without using sedation if they do not sleep. Your decision will not affect your involvement in the DCHLS and will not change your follow up or routine care in anyway.

**What are the possible benefits to your child?**

Although the infant lung function test will not be of any immediate benefit for your child it will record your babies' lung growth and how well the lungs are working through the 2 years of the study. This may be useful to your baby in the future if they should get a lung disease. The tests will also allow us to understand what is normal lung growth and function for babies in South Africa. This is important so we can better understand what causes damage to babies' lungs in South Africa. In this way the study will help us to better understand the causes of illness in children, and identify the things that may harm their health. We hope that this will lead to improvements in child health.

**What are the possible risks to your child?**

The lung function tests have no serious risk to your baby. The test is done with a mask over the babies' nose and mouth while they are sleeping. They breathe air through the mask as they would normally. Sedation with chloral hydrate does have risks, the most serious being slowing of breathing or making the heart beat irregularly. This may happen if a very large dose or repeated doses are given. There is only a very small chance that this would happen with the standard dose used in this study. We will not repeat a dose

on any testing day. Your child will be monitored and a trained medical professional will always be present.

**Who will do the testing on my infant?**

There is a team of trained staff who will do the testing and help with all the study related questions and other tests on the day. For the lung function testing the team is: Dr Diane Gray (child respiratory specialist), Ms Ane Alberts (respiratory technologist) and Sr Lauren Willemse (professional nurse and co-ordinator of this section of the study). They have all been trained in doing this testing.

**Will I find out the results of the test?**

Yes, within 2 weeks of testing a feedback letter should be sent to you. If your child is found to have an abnormal lung function test they will be referred by our team to the paediatric doctors at Paarl hospital for further assessment and any necessary treatment. Please check that the ILF study co-ordinator, Sr. Lauren Willemse has your correct address when you come for the test.

**What do I do if I have any questions?**

If you have any questions about this study, you can ask study staff or contact Lauren Willemse or Diane Gray at 021 860 2802. For questions about your rights as a study participant call the Research Ethics Committee, University of Cape Town, telephone: 021-4066492.

I_____

the parent of _____

have read and understood this form. My questions have been answered. I voluntarily consent to my child having an Infant lung function test. In addition I consent/do not consent (delete where applicable) to sedation for the 1 and 2 year testing if it is necessary.

Signature: _____ Date: _____

# Appendix B

## Drakenstein Child Lung Health Study – Infant lung function testing

### Parent information sheet

Thank you for agreeing to be part of this study. This information sheet aims to explain what the infant lung function is for and what it entails for you and your baby.

### Why is infant lung function being measured?

South African children have a high rate of chest problems such as pneumonia, asthma and cough. We know from studies done in other countries that there are many things that can be a risk for chest problems in young children such as poor growth, exposure to cigarette smoke and other air pollutants and infections. At present we do not know what the most important risks are for South African children nor what is normal lung function for South African babies as it has not been measured before. By measuring a baby's lung function over time we can find out how big their lungs are, how well the lungs work and if they grow normally over time. This study will allow us to find out what is normal lung function in South African babies and will provide important information about what causes chest disease in children and the effect these things have on lung growth and later chronic chest problems. This will help us to understand how best to prevent and treat lung disease in our children.

### What does infant lung function measure?

The test measures your baby's lung size and breathing pattern. There are 4 different tests, but some are done at the same time.

### Tidal breathing flow volume test

The first test measures how fast (flow) your baby breaths and how much (volume) they breathe with each normal breath. By doing this the equipment can calculate information about the growth and working of your babies lungs.

**Exhaled nitric oxide test**

At the same time the equipment can measure the amount of a gas called nitric oxide (NO) that is in your baby's breath when they breathe out. NO is a gas that is important in the working of some of our bodies functions. We all have NO in the air we breathe out. High levels of NO in our breath can mean our airways or breathing tubes are inflamed. This has been shown to happen with diseases such as asthma or exposure to pollutants such as cigarette smoke.

**Sulphur hexafluoride (SF6) multiple breath washout test**

We can also measure the size of your baby's lungs and the way the different parts of the lungs contribute to breathing. We do this by adding an inert gas (gas that does not get absorbed into the body) called SF6 to the air the baby breaths for a short period. This gas is not smelly or dangerous to your baby.

**Forced oscillation technique**

This test uses sound waves to test how the baby's lungs function and can pick up early problems with infants lung growth and function.

**How is the test done?**

All the tests are done during quiet normal breathing (tidal breathing) when your baby is asleep. Your baby will need to be asleep and, while lying on his/her back, a face mask with air flowing through a flow meter will be placed over your baby's nose and mouth. This can take all the measurements mentioned above. Often babies wriggle, sigh or wake and so sometimes we will need to repeat the test. We need to try and get 3 good tests. Depending on how deeply baby is sleeping it can take anywhere from 20 min to 1 hour.

As mentioned the test can be done during normal sleep for babies. However for the testing at 1 and 2 years of age, if your child is unable to stay asleep for the test we may need to give your baby some sedation or medicine that will make your baby sleepy. This medication is called chloral hydrate and is used commonly for this reason around the world. The benefit of this medication is that it can help your child sleep without signifi-

cantly affecting his/her breathing pattern. If your child requires sedation he/she will need to wait for the sedation to wear off (1-2 hours) before you can go home. There will always be a nurse or doctor with your baby during testing (with or without sedation).

**Who will do the testing on my infant?**

There is a team of trained staff who will do the testing and help with all the study related questions and other tests on the day. For the lung function testing the team is: Dr Diane Gray (child respiratory specialist), Ms Ane Alberts (respiratory technologist) and Sr. Lauren Macmillan (registered nurse and co-ordinator of this section of the study). You can contact them on: 021 860 2802 if you have any concerns or questions.

**Will I find out the results of the test?**

Yes, within 2 weeks of testing a feedback letter should be sent to you. Please check that the ILF study co-ordinator, Sr. Lauren Macmillan has your correct address when you come for the test.

**What if I have questions about the infant lung function study?**

If you have any questions about this study, you can ask the study staff or contact Lauren Willemse or Diane Gray of the infant lung function team at Paarl Hospital, telephone: 021 860 2802. For questions about your rights as a study participant call the Research Ethics Committee, University of Cape Town, telephone: 021-406 6492.

## APPEDNIX C

UNIVERSITY OF CAPE TOWN

**Faculty of Health Sciences**
**Faculty of Health Sciences Human Research Ethics Committee**
**Room E52-24 Groote Schuur Hospital Old Main Building**
**Observatory 7925**
**Telephone** [021] 406 6338 • **Facsimile** [021] 406 6411
**e-mail:** sumayah.ariefdien@uct.ac.za

**04 December 2012**

**HREC REF: 423/2012**

**Prof H Zar**
Paediatrics
Red Cross War Memorial Children's Hospital
Rondebosch

Dear Prof Zar

**PROJECT TITLE: DRAKENSTEIN CHILD LUNG HEALTH STUDY - INFANT LUNG FUNCTION SUB- STUDY (LINKED TO HREC 401/2009)**

Thank you for addressing the issues raised by the Human Research Ethics Committee.

It is a pleasure to inform you that the HREC has **formally approved** the above mentioned study.

**Approval is granted for one year till the 28 December 2013.**

Please submit a progress form, using the standardised Annual Report Form, if the study continues beyond the approval period. Please submit a Standard Closure form, if the study is completed within the approval period.

Please supply the SOP for Child sedation as per your response for the HREC's records.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

**Please quote the HREC. REF in all your correspondence.**

Yours sincerely

Signed by candidate

**PROFESSOR M BLOCKMAN**
**CHAIRPERSON, HSF HUMAN ETHICS**

Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938

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# APPENDIX D

**Table of Cohort Demographics for infants born on the Drakenstein Child Health Study, N=1139 (including 4 twins and 1 triplet)**

| | Black African (Mbekweni) | Mixed race (Newman) | Total | P-value |
|---|---|---|---|---|
| Male sex N (%) | 312 (50) | 279 (52) | 591 (52) | 0.112 |
| Weight-for-GA (WAZ) med (IQR) | -0.4 (-1.2 to 0.2) | -0.7 (-1.4 to -0.1) | -0.6 (-1.4 to -0.1) | 0.002 |
| Length-for-GA (HAZ) med (IQR) | 0.1 (-0.9 to 1.0) | 0.0 (-0.9 to 0.8) | 0.0 (-0.9 to 0.9) | 0.156 |
| Head circumference-for-GA (WAZ) med (IQR) | -0.4 (-1.2 to 0.4) | -0.6 (-1.4 to 0.2) | -0.5 (-1.3 to 0.3) | 0.013 |
| Preterm (<37 week) N (%) | 108 (17) | 82 (16) | 190 (17) | 0.586 |
| Breastfeeding initiated after birth N (%) | 373 (77) | 398 (96) | 771 (86) | <0.001 |
| | | | | |
| Maternal smoking | 573 (54) | 482 (46) | 1055 | |
| Active N (%) | 89 (16) | 253 (53) | 342 (32) | <0.001 |
| Passive N (%) | 293 (51) | 173 (36) | 466 (44) | |
| None N (%) | 191 (33) | 56 (12) | 247 (23) | |
| | | | | |
| Maternal HIV positive N (%) | 227 (37) | 17 (3) | 244 (22) | <0.001 |
| | | | | |
| Socioeconomic status | | | | |
| Lowest quartile N (%) | 190 (31) | 87 (17) | 277 (24) | <0.001 |
| Low-moderate quartile N (%) | 156 (25) | 133 (26) | 289 (25) | |
| Moderate-high quartile N (%) | 153 (25) | 136 (27) | 289 (25) | |
| Highest quartile N (%) | 122 (20) | 156 (30) | 278 (25) | |